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ENDOTHELIN-1 AND RADIATION-ASSOCIATED IMPOTENCE

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment the requirements for the degree of Master of Science

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Abstract

The mechanism by which radiation treatment for prostate cancer induces erectile dysfunction remains poorly understood. The literature is replete with studies demonstrating the association between radiation and impotence, however, little insight into the true pathogenesis exists. The pathophysiology of this complication has been linked mainly to injury to the internal iliac and penile arteries. Current theories describe radiation causing an accelerated atherosclerosis in these vessels. Recently, an important role has been atttributed to endothelin-1 (ET-1), a potent vasoconstrictor and a mitogenic factor. The prostate and the vascular endothelium, including that of the penis, are known to produce ET-1. Furthermore, it has been shown that there are two receptor subtypes in the penis, ET_a and ET_b. Radiation treatment is known to cause an increase in ET-1 and may be a fundamental cause of radiation-associated impotence (RAI).

The aim of the present study was to evaluate the role of ET-1 in RAI. In the first part of our study, rats were divided into groups according to a different radiation dose. A ET-1 time course in tissue and serum was established. Markedly higher ET-1 levels were found in a high radiation dose compared with a lower dose and in the control group.

In the second part of the study, rats underwent evaluation of erectile function after radiation therapy. Subsequently, rats were given an antagonist of ET_a (BQ-123) to assess whether the erectile response could be potentiated. Our results show that the use of this specific antagonist results in an improved erectile activity in a rat animal model.

Our preliminary results show that radiation treatment upsets the delicate balance of vasoactive substances in the corpora cavernosa. This increase in ET-1 may cause either increased corporal smooth muscle contractility or impaired corporal smooth

muscle relaxation. This increase of ET-1 may be responsible for the atherosclerotic development and gradual loss of erection seen in irradiated patients.

Résumé

Le méchanisme selon lequel la radiothérapie dans le cadre du cancer de la prostate induit des troubles de l'érection reste mal compris. Les publications sur la question fourmillent d'études qui démontrent le rapport entre la radiation et l'impuissance, sans toutefois approfondir la question de sa pathogenèse. La pathophysiologie de ce trouble est principalement liée à des lésions au niveau des artères pénienne et iliaque internes. Les théories actuelles veulent que l'irradiation accélère l'athérosclèrose de ces vaisseaux. Dernièrement, un rôle important a été attribué à l'endothéline (ET-1), puissant vasoconstricteur et facteur mitogène. La prostate et l'endothélium vasculaire, y compris celui du pénis, sécrètent de l'ET-1. En outre, on a démontré que le pénis abrite deux sous-types de récepteurs, à savoir ET_a et ET_b. La radiothérapie provoque une augmentation des concentrations d/ET-1 qui pourraient bien être la cause fondamentale de l'impuissance liée à la radiothérapie.

L'objectif de cette étude est d'évaluer le rôle de l'ET-1 dans l'impuissance liée à la radiothérapie. Dans la première partie de l'étude, des rats ont été divisés en différents groupes, en fonction de la dose d'irradiation qui leur était administrée. On a ensiute suivi l'évolution des concentration d'ET-1 dans les tissus et le sérum. Plus la dose d'irradiation était élevée, plus les concentrations d'ET-1 étaient importantes, par rapport aux doses plus faibles et au groupe témoin.

Dans la deuxième partie de l'étude, la fonction érectible des rats a été évaluée après la radiothérapie. On leur a ensuite administré un antagoniste d'ET_a (BQ-123) pour déterminer si la réponse érectible pouvait faire l'objet d'une potentialisation. Nos résultats démontrent que l'administration de cet antagoniste spécifique améliore la fonction érectile du modèle animal.

Nos résultats préliminaires révèlent que la radiothérapie perturbe l'équilibre délicat des substances vasoactives dans la corpora cavernosa. L'augmentation d'ET-1 peut provoquer une contractilité accrue du muscle lisse corporel ou nuire à sa

relaxation. Cette augmentation d'ET-1 pourrait fort bien être la cause de l'apparition de l'athérosclérose et de l'anérection chez les patients irradiés.

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List of abbreviations

CaM: Calmodulin

CGRP: Calcitonin-gene related polypeptide

ECE: Endothelin converting enzyme

EDRF: Endothelium-derived relaxing factor

eNOS: Endothelial nitric oxide synthase

ET-1: Endothelin-1

FAD: Flavin adenine dinucleotide

FMN: Flavin mononucleotide

ICP: Intracavernosal pressure

iNOS: Inducible nitric oxide synthase

L-NAME: N^G -nitro arginine methyl ester

MPG: Major pelvic ganglion

NADPH: Nicotinamide adenine dinucleotide phosphate

NE: Noradrenaline

nNOS: Neuronal nitric oxide synthase

NO: Nitric oxide

NOS: Nitric oxide synthase

NPT: Nocturnal penile tumescence

PAI-1: Plasminogen activator inhibitor-1

PKAP: Protein kinase phosphorylation site

PKC: Protein kinase C

RAI: Radiation-associated impotence

SEM: Standard error of the mean

STX: Sarafotoxin

TFA: trifluoric acid

t-PA: tissue plasminogen activator

VIC: Vasoactive intestinal contractor

Thesis of Scott Merlin

Please note an error in pagination. Between pages 20 and 21, there is a page with Figure 2. This page is not numbered. All other pages with figures are numbered and correct.

INTRODUCTION

1.1-Impotence: A widespread clinical problem

Impotence can be defined as the consistent inability of a man to achieve and maintain a sufficiently rigid erection for sexual intercourse. It is estimated that 30 million North American men between 40 and 70 years of age suffer from this dysfunction[1,2] . Although erectile dysfunction is often an age-related problem, it can be caused by a number of factors; classified as vasculogenic, neurogenic, psychogenic, cavernosal or hormonal[2]. Erectile dysfunction can result from systemic illnesses and drugs which can be concomitant and causative. Among the major causes of physical impotence are the following: diabetes, cardiovascular problems, radical pelvic surgery, injuries and hormone inadequecies.

1.2- Insight into the etiology of vasculogenic impotence

Penile erection and detumescence primarily are controlled by hemodynamic events of which increased arterial inflow is of chief importance. The process of penile erection is therefore dependent on an adequate arterial supply. Arterial insufficiency may result from congenital anomalies or trauma, but the majority of cases can be attributed to a generalized process of atherosclerosis[3]. Atherosclerotic lesions are characterized by the proliferation of smooth muscle and deposition of lipid in the vessel wall[4]. Associated risk factors include hypercholesterolemia, cigarette smoking, diabetes, hypertension, radiation exposure, and perineal trauma leading to direct vessel injury.

In normal vessels, endothelial cells provide a mechanical barrier for underlying smooth muscle cells, blood cells and other circulating substances; they produce local

factors that act in a paracrine fashion and are able to alter vascular tone and induce growth. Some examples include endothelin-1 and angiotensin II which are powerful vasoconstrictors that tend to promote smooth muscle proliferation; these agents are balanced by vasodilators such as nitric oxide (NO), and prostaglandins, which inhibit smooth muscle growth[5].

Any alteration of vasoconstrictor and vasodilator balance can lead to the modification of endothelial function, which may ultimately result in the induction of smooth muscle proliferation and collagen deposition in the vessel wall.

1.3-Radiotherapy treatment

The aim of radiotherapy treatment is to sterilize all of cells of the tumour while avoiding damage to normal cells beyond their repair capabilities.

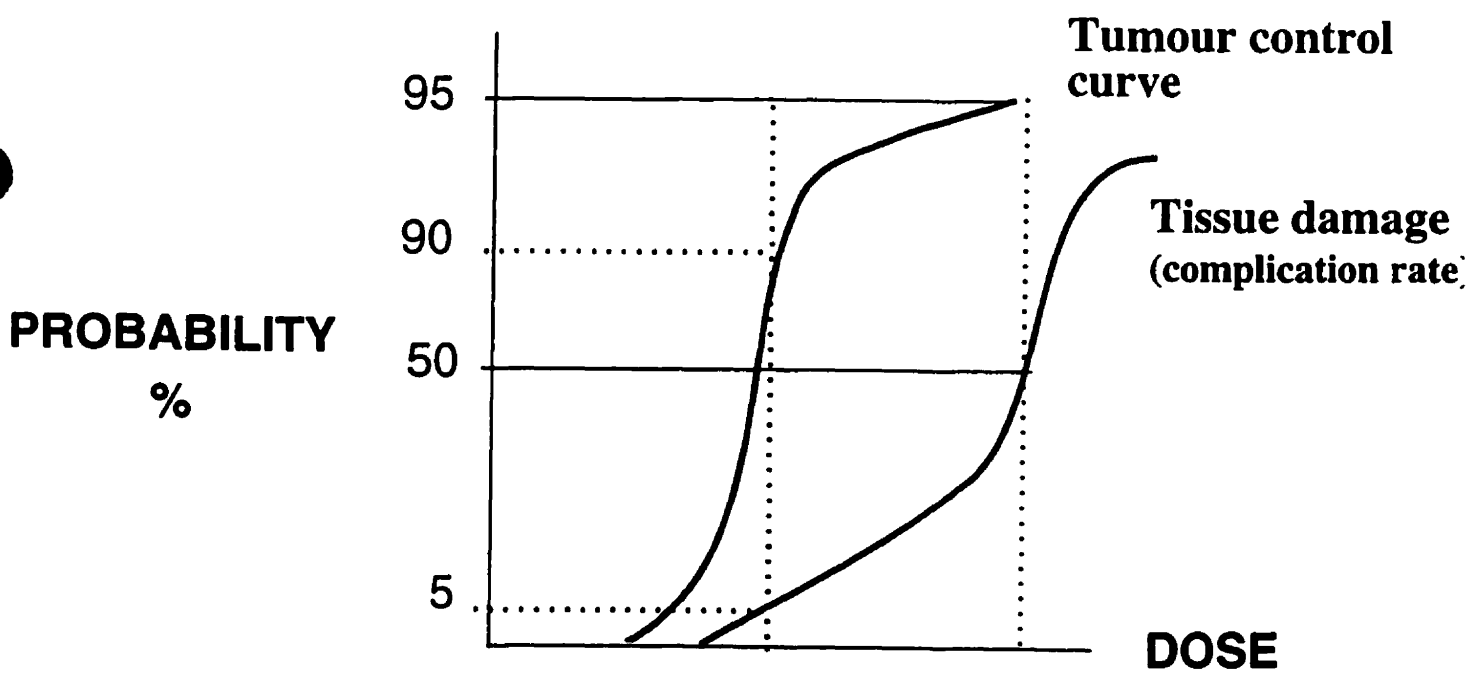

Figure.1: Sigmoid curves of tumor control and complications.**A**. Dose for tumor control with minimum complications. **B**. Maximum tumor dose with significant complications. Taken from Ref. 7

Figure 1 shows the possible benefit derived from an idealized radiation treatment, allowing minimal complications. As you increase the dose of radiation, you increase the probability of tumour control. However, this is a steep curve so a slight increase in dose results in a strong increase in tumour control. In very close proximation to the tumour control curve, you have the tissue damage control curve which has the same sigmoidal shape. Thus, a slight change in dose has significant effect on the complication rate. The accepted convention is to aim for a 90% tumour control with a 5% complication probability[6].

The biological targets of radiation are the cells of the body's various tissues. Radiation therapy is delivered in the form of electromagnetic waves such as X-rays or gamma rays. X-rays are generated by linear accelerators. They are ideal for treatment of tumours, as they penetrate to great depths before reaching full intensity and thereby sparing toxicity to skin.

The specific target of radiation damage is DNA[7] (Figure.2). Radiation exerts its biological effect by ejecting electrons from target molecules, a process called ionization. Ionizing radiation may interact with DNA directly or indirectly. Direct action involves the ionization of atoms in the cell nucleus. More important in radiotherapy, is the indirect action on the cell nucleus; X-rays interact with other molecules, such as water, forming free radicals. The free radicals are highly reactive and cause breaks in chromosomes. The biological end point is refelected by the loss of cellular reproductive capacity. The most common damage is the single-stranded DNA break. When the cells go into mitosis, they will die because of the damaged DNA. This is called reproductive capacity.

1.4-Advantages and Limitations of Radiotherapy

Advantages	Limitations
preservation of organ function	local form of therapy
better cosmesis	radioresistance
avoidance of surgery	radiation therapy side effects
effectiveness against a wide range of malignancies	prolonged treatment time

1.5-RADIATION-ASSOCIATED IMPOTENCE

Prostate cancer has recently gained the unenviable position as the most common solid cancer among males. Radiation therapy to the pelvis and prostatic fossa is a current mainstay of therapy, and 5000 Canadian men are planned to undergo therapy this year[8]. Erectile dysfunction is a known side effect following irradiation. The literature is replete with studies demonstrating the association between radiation and impotence, however, little insight into the underlying pathogenesis is known. The pathophysiology of this complication has been linked mainly to blood vessel damage and/or damage to the nerves that innervate the corporal smooth muscle.

The basic therapeutic objective in prostate cancer is a curative approach. However, in these patients, loss of sexual function is common and should be a major concern in choosing the treatment approach. The association between erectile dysfunction and radiation has been reported in a number of studies[9-13]. The percentage of radiation-associated impotence (RAI) varies from 22 to 84%[14-15]. Two

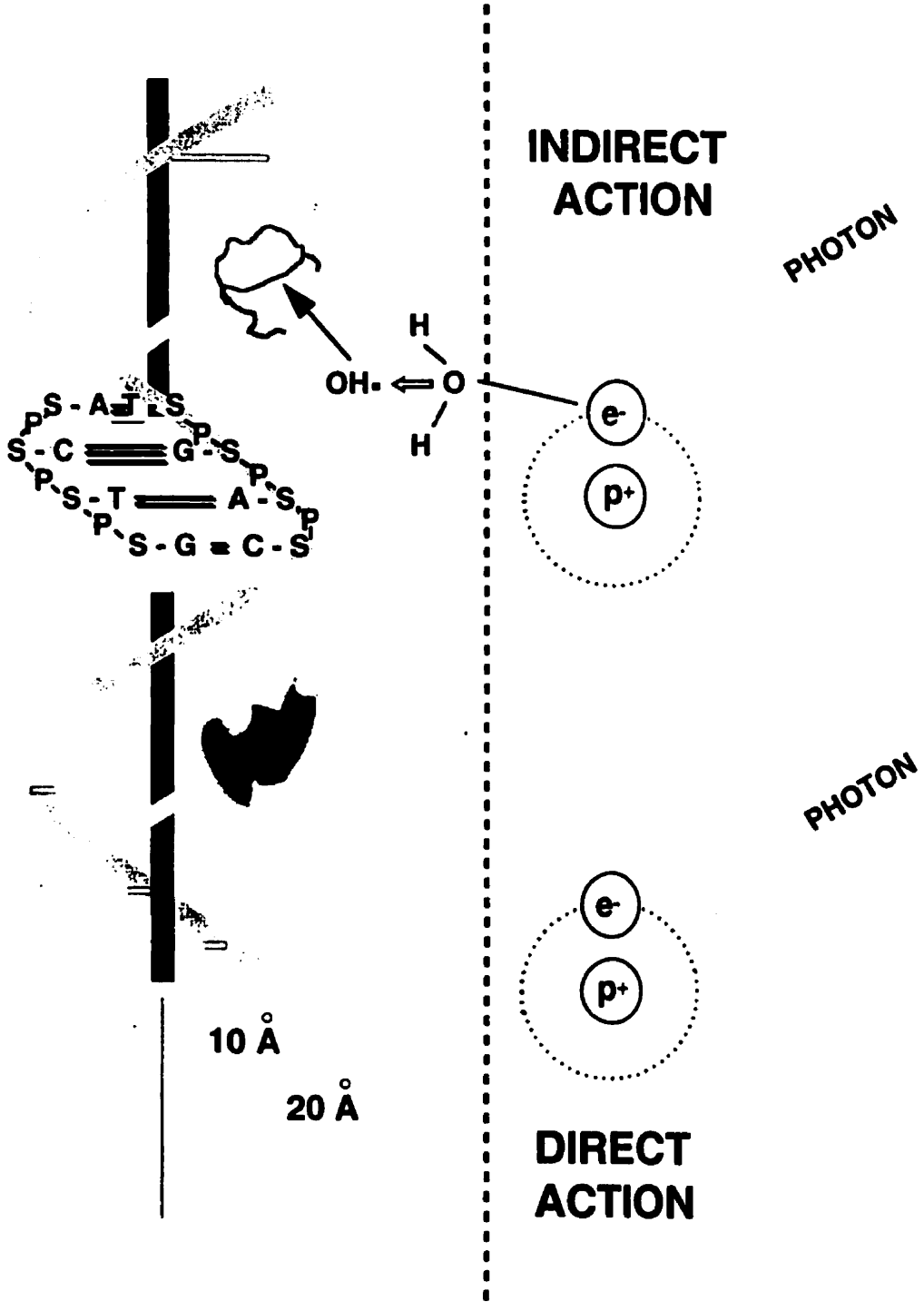

Fig 2. Two mechanisms of DNA damage; direct and indirect ionization

characteristics of RAI include it's variable onset and irreversibility. RAI can occur immediately after radiation therapy, however it has also been reported to occur after many years following treatment. Importantly, once RAI manifests, it is usually progressive and irreversible.

The mechanism of RAI remains unknown. Speculative explanations include damage to blood, nerves, and testicular injury.[15-16] The underlying pathophysiology has classically been linked to vascular causes as vasculogenic impotence is the most consistent form of organic impotence reported after prostatic irradiation[14-15]. Goldstein[14] and colleagues reported that 70% of their patients with prostate cancer were potent. After radiation therapy, only 13% remained potent. In those patients whose erectile capacity changed, 60% had resting Doppler values that fell significantly with exercise, implying substantial vascular pathologic features in the hypogastric-cavernous arterial bed. Occlusive vascular disease within the pelvic radiation field was confirmed. Bilateral narrowing of the internal iliac arteries, tortuosity and occlusions of the internal pudental and penile arteries were observed.

In the presence of other vascular risk factors, radiation may predispose the patient to the development of pelvic vascular insufficiency. Goldstein et. al. suggested that there be a relation with the total amount of radiotherapy delivered to the patient, which might act synergistically with vascular risk factors (cigarette smoking, hypertension, high fat food intake, family history, and diabetes) to accelerate atherosclerosis, with the resultant atherosclerosis inducing RAI.

The possibility that RAI has a vasculogenic component is supported by both animal and clinical studies. Warren [17] reported endothelial degeneration of the elastic membrane and fibroblastic proliferation of the media as a result of radiation to the arteries. Lindsay[18] and colleagues subjected dogs to localized aortic irradiation and observed the development of atheromatous plaques. Other studies have shown that hypercholesterolemic diets which may act synergistically with radiation therapy in

producing atherosclerosis[19-20]. In addition, there exists many clinical reports in which radiation-associated complications were suggested to have a vasculogenic etiology[21-22]. Swanson[15] et. al. suggested that pelvic irradiation might cause injury to both pelvic nerves and blood vessels. Injury to the pelvic nerves could not be identified, however, vascular disease was identified in all patients whose erectile capacity changed after radiation therapy. Portions of the internal pudendal and penile arteries are inevitably exposed to radiation delivered to the prostate.

Papaverine is a direct smooth muscle relaxant that enables the evaluation of the integrity of the vascular supply. Carrier[23]et. al. showed a poor response to papaverine in a rat model irradiated at a high dose over the prostatic bed. Their findings support the theory that RAI has a vasculogenic component.

Radiation therapy has been shown to affect components of the nervous system responsible for erection. It is now known that penile erection is mediated primarily through the non-adrenergic, non-cholinergic nervous system. Nitric oxide has become widely accepted as an important transmitter of penile erection[24-26]. Carrier et. al.evaluated the effect of radiation therapy on nitric oxide synthase- containing nerve fibers in the rat penis. Histologic evaluation demonstrated that, with an increasing dose of radiation, the number of nitric oxide synthase-containing nerve fibers decreased significantly.

Normal erectile function is characterized by a delicate balance *in vivo* between the effects of vasoconstricting and vasorelaxing agents on the level of corporeal smooth muscle tone[27-28]. Any imbalance can lead to erectile dysfunction. Based on the observations of Carrier et. al., an imbalance does indeed exist, which may leave the effects of vasoconstrictors prevailing.

In summary, the reduced erectile function observed in many men following prostatic irradiation is likely multifactorial. Based on data obtained from the current literature, it appears that a vasculogenic component is the most consistent organic

abnormality determined in patients with RAI. Further, it seems likely that a paracrine mediated change to local vasomotor tone may play a role in RAI. Our hypothesis is that endothelin-1 may play a significant role in this increased tone following RAI.

1.6.-Anatomy of erection

1.6.1 Penile Anatomy

The penis is composed of erectile tissue enclosed in three cylindrical fibrous compartments: the two corpora cavernosa and the corpus spongiosum (Figure.3). The corpus spongiosum surrounds the penile urethra and terminates as the glans penis. The corpora cavernosa are side by side and joined medially for the distal three quarters of their length. Proximally, they seperate to form the crura, which are tapering fibrous structures. Each corpus cavernosum is surrounded by a thick, semi-rigid, fibrous sheath known as the tunica albuginea, which is a bilayered structure with multiple sublayers, consisting mostly of collagen fibers, and some elastic fibers . Histologically, the paired corpora cavernosa consist primarily of bundles of smooth muscle cells within a collagenous extracellular matrix. The corpora cavernosa smooth muscle cells form a framework of blood filled sinuses called lacunar spaces. These spaces get filled with blood to allow for the engorgement of the penis during tumescence.

The blood supply to the corpora cavernosa is provided by the paired cavernous arteries which are terminal branches of the internal iliac arteries. The cavernosal artery, a narrow vessel, enters at the base of the penis along with the cavernosal veins and nerves. Upon entering the corpora cavernosa, it divides into smaller arterioles called the helicine arterioles which provides blood supply to the lacunar spaces. The corporal bodies are drained by the emissary veins that pierce through the tunica albuginea and drain into the deep dorsal vein.

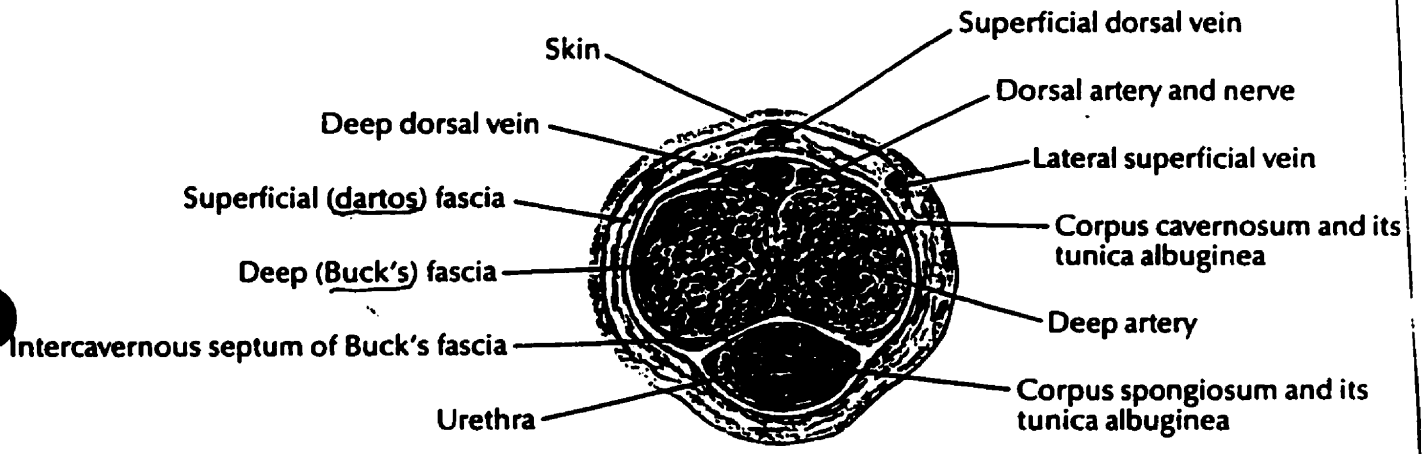

Figure 3. Cross section of the human penis.

1.6.2 Radiation Anatomy

The radiation field delivered to the prostate is approximately a 6x7 cm field. Within this field, the internal pudendal (hypogastric artery) and penile arteries are exposed to approximately 70 to 75% of radiation dose delivered[14]. As a result, there is opportunity for radiation-associated vascular disease to develop, which is enhanced by the presence of previous vascular risk factors.

. More important, stenoses and occlusions in these vessels restrict necessary increase in blood flow delivery to corporal bodies at time of sexual stimulation.

1.7-Physiology of erection

Penile erection is a complex neurovascular phenomenon which includes three major events: increase in arterial flow into the penis, smooth muscle relaxation, and a decrease in venous outflow. However, this complex physiological event also requires the participation of neurotransmitters, striated muscle, and the tunica albuginea.

In the flaccid state, only a small amount of blood enters the penis, for nutritional purposes. The cavernosal arteries, and helicine artrioles are constricted. Under the proper erotic or tactile stimulation, nerve impulses travel from the spinal cord via nerve fibers of the pelvic plexus and causes the release of neurotransmitters. This neurotransmitter release causes vasodilation of the cavernosal arteries and helicine arterioles, and the relaxation of the cavernosal smooth muscle[29]. This results in as much as a 60-fold increase in the blood flow into the corpora cavernosa As the lacunar spaces fill with blood, they compress the subtunical venules against the tunica albuginea, and venous outflow is sufficiently decreased to result in turgidity of the corpora cavernosaand corpus spongiosum. This mechanism is referred to as the veno-occlusive mechanism. Contraction of the ischiocavernosal and bulbourethral muscles allows for further rigidity of the penis.

The mechanism of detumescence is caused by activation of the sympathetic nervous system. The release of noradrenaline causes an increase in helicine and smooth muscle tone. This leads to a decrease in arterial flow, and contraction of the lacunar spaces. As a result, venous outflow will increase. Recent studies have suggested that endothelin-1, a potent vasoconstrictor, released from endothelial cells, may be one of the principle factors responsible for the local control of penile flaccidity[30-31]. This will be reviewed in more detail in section 10.15.

1.8-Neural basis of erection

1.8.1 Neural pathways mediating erection

In addition to the advances in the undertanding of the hemodynamics of erection, great strides have been achieved in our knowledge of the neural pathways mediating erection. Three major neural pathways inducing erection have been described[6]:

1) *the reflexogenic:* responsible for the induction of erection through direct stimulation of the genitalia.

2) *the psychogenic:* capable of inducing erections based on fantasy, gustatory, or audiovisual stimuli.

3) *the nocturnal*: which occurs mostly during rapid-eye-movement (REM) sleep; this mechanism remains poorly understood[32].

1.8.1.1-Reflexogenic neural pathway

In reflexogenic erections, sensory impulses coming from penile skin and the penile glans are carried by the dorsal nerve of the penis, pudendal, and sacral nerves to the spinal cord. Reflexogenic erections are controlled by nerves of parasympathetic, sympathetic, and somatic origin.

A) Parasympathetic nervous system

Efferent nerves originating from spinal segments S_2 to S_4 are commonly known as the sacral erection center and are part of the parasympathetic nervous system[33,34]. These nerve fibers form the pelvic nerves (also known as the nervi erigentes) which join the pelvic plexus providing parasympathetic neural supply to the pelvic organs and external genitalia[35].

B) Sympathetic nervous system

In addition to parasympathetic neural supply, the sympathetic nervous system innervates the urogenital system. Sympathetic nerve fibers which originate from the intermediolateral gray matter of the eleventh thoracic to second lumbar spinal segments project to the pelvic plexus as well. In addition, there exists sympathetic fibers originating from the sacral sympathetic chain ganglia which join the pelvic nerves. Through its activation, the sympathetic nervous system is known to inhibit erections produced by stimulation of the cavernous nerves by its release of noradrenaline[36,37].

The pelvic plexus, which is formed by both parasympathetic and sympathetic nerve fibers provides neural supply to the bladder, seminal vesicles, prostate, rectum, membranous urethra, and corpora cavernosa. Those nerves originating from the pelvic plexus which innervate the cavernosal bodies of the penis are termed the cavernous nerves and they are responsible for the neuroregulation of erection (Figure.4). In an earlier study by Walsh and Donker, cavernous nerves were traced in male fetuses and stillborn neonates[39]. This study identified that the cavernous nerves were situated between the prostate and rectum running on the dorsolateral aspect of the prostate.

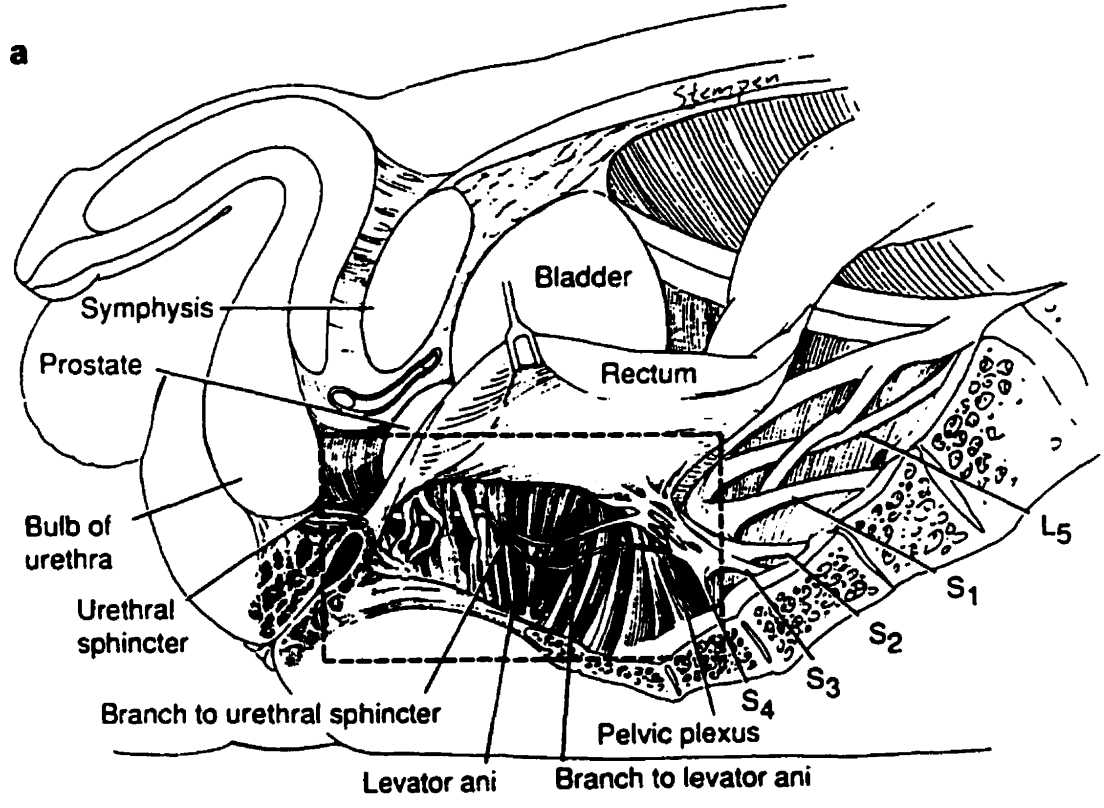

Figure 4. Pelvic nerves originating from S_2 to S_4 form in part the pelvic plexus.

C) Somatic nervous system

The somatic nervous system plays an esential role in preserving normal erectile function. It is required to achieve full rigid erections[39]. Motor neurons arising from Onuf's nucleus located in the second to fourth sacral spinal segments control bulbospongiosus and ischiocavernosus muscle tone allowing them to produce full rigid erections[40].

1.8.1.2-Psychogenic neural pathway

In contrast to reflexogenic erections, which are primarily controlled at the spinal cord level, psychogenic erections result from fantasy, gustatory, or audiovisual stimuli and are mainly controlled by the brain. Facilitory or inhibitory impulses which are integrated in the limbic system and medial preoptic anterior hypothalamic area descend via the ventrolateral area of the medulla and pons to the sacral and lumbar spinal centers whereby they activate or inhibit the erectile process. It is believed that the thoracolumbar sympathetic pathway is responsible for carrying these impulses to spinal centers[41]. In addition to being strong inducers of erection, psychogenic stimuli can enhance erections induced through the reflexogenic pathway. There appears to be a synergistic effect between the reflexogenic and psychogenic pathways[33,42]. Psychogenic stimuli as well have the ability to block erection. There are two possible mechanisms by which this inhibition may be produced:

1) direct inhibition by the brain to the spinal centers.
2) excessive sympathetic outflow or elevated peripheral catecholamine release resulting in a decrease in smooth muscle relaxation[43].

1.8.1.3-Nocturnal neural pathway

Nocturnal penile tumescence (NPT) was first described by Halverson in 1940 in infants[44]. Subsequently, the same phenomenon was described in adults by Ohleyer and associates[45]. Although the nocturnal neural pathway remains poorly understood, it is known that NPT is a naturally occurring, non-sexually stimulated phenomenon whose neural pathway is believed to be similar to that of sexually stimulated erection[46]. NPT occurs most often during rapid eye movement sleep but however, can occur during non-rapid eye movement sleep.

1.9 The rat as an animal model for the study of erectile dysfunction

Most of the animal studies investigating the hemodynamics and neuroanatomical basis of erection have been carried out on the dog and monkey. However, since its first description by Quinlan et al., the rat model for erection has become more frequently used.[61] Anatomical dissections using this rat animal model have demonstrated the existence of a pelvic plexus bilaterally which in the rat is referred to as the major pelvic ganglion (MPG). The MPG is located on either side of the dorso-lateral lobes of the prostate. This ganglion receives neural supply from the sixth lunbar (L6) and first sacral (S1) spinal nerves via the hypogastric nerve. Branches from the MPG innervate the bladder, prostate, distal ureter, rectum, vas deferens, and urethra. In addition, the MPG gives rise to a large nerve fiber (known as the cavernous nerve) which runs on the lateral aspect of the urethra, penetrates the urogenital diaphragm, and provides neural supply to the erectile bodies (corpora cavernosa). As in the human, the cavernous nerve has been shown tobe responsible for the neuroregulation of erection.

1.9.1.1-Nitric oxide and penile erection

It is now known that penile erection is mediated primarily through the non-adrenergic, non-cholinergic nervous system. Several putative neurotransmitters have been suggested to be mediators of erection[47-49]. It is now accepted that nitric oxide (NO) is the principal mediator of erection. NO is a noxious free radical gas that has been implicated as a transmitter in neural pathways to the lower urogenital tract.[50-52] NO is formed from L-arginine with the products of this reaction being NO and citrulline. This reaction requires the presence of molecular oxygen (O_2) and of nicotinamide adenine dinucleotide phosphate (NADPH).

1.9.1.2-Nitric Oxide Synthase

The enzymes which are responsible for catalyzing the synthesis of NO are referred to as nitric oxide synthases. Nitric oxide synthases (NOS) have had a significant impact in the study of NO's role in mammalian systems as direct measurement and localization of this transmitter is hampered due to its gaseous nature and short half-life.

The synthesis of NO in mammalian systems was first demonstrated in vascular endothelium, brain tissue and activated macrophages[53]. It was only recently however through cloning[54] that NOS has been confirmed to exist in three distinct forms: (Figure.5)

1) neuronal NOS was first identified as neuronal constitutive NOS also known as type I NOS[55].
2) endothelial NOS was first identified as constitutive in vascular tissue also known as type III[56]

3) inducible NOS was first identified as being inducible by cytokines in macrophages and hepatocytes also known as type II NOS[57].

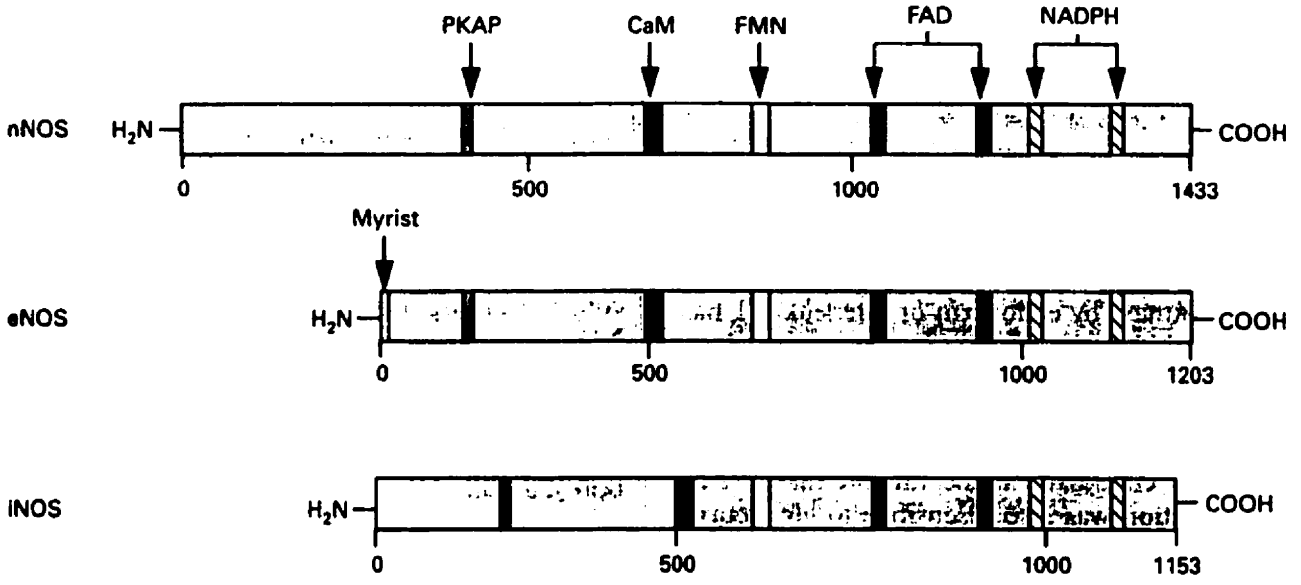

Figure 5- Primary structure of the different isoforms of the human NOS enzymes. The lenght and binding sites for NADPH, FAD,FMN, calmodulin (CaM), as well cyclic AMP-dependent protein kinase phosphorylation site (PKAP), and N-terminal myristoylation site (Myrist) are indicated.

The localization of nNOS to neuronal fibers innervating the corporal bodies and blood vessels of the penis have suggested a possible involvement of NO as a mediator of penile erection.[58,59] Futhermore, it was shown that several NOS inhibitors, such as L-NAME and the selective nNOS inhibitor, 7-Nitroindazole, blocked cavernosal smooth muscle relaxation, and caused a dose-dependent decrease in intracavernosal pressure in a rat animal model, respectively.[60,61] Therefore, the selective localization and in vitro and in vivo blockade of NOS has had a significant impact in implicating NO as an important neuronal mediator of penile erection.[47,49]

ENDOTHELIN

1.11.1-Introduction

Within the past few decades, there is an increasing body of evidence that the vascular endothelium plays an important role in the regulation of vascular tone. This concept first emerged after the discovery of endothelium-derived relaxing factor (EDRF) in the 1980, which is known to be nitric oxide. However, less attention was paid to the endothelium-derived constricting factor until the mid-1980s. In 1985, Highsmith and his colleagues[63] reported the existence of a vasoconstrictive factor in a conditioned medium of cultured bovine endothelial cells. They suggested that the factor was a peptide since it was sensitive to trypsin. In 1988, Yanagisawa and colleagues[64] isolated, sequenced, and cloned this peptidergic EDCF, and named it Endothelin (ET). This paper stimulated worldwide interest and unprecedented research activity. The discovery of the ET peptide family initiated a new field of biomedical research which promises to lead to a better understanding of the pathophysiology of several diseases and to the development of novel therapeutics.

The 21 amino acid peptide has no similarity in its sequence to the known peptides of mammalian origin. However, the sequence of a rare snake venom, Sarafotoxin (STX), was reported to be very similar to that of ET[65]. Furthermore, a feature similar to ET is also shared by another peptide whose gene has been identified in the mouse genome and expressed in the intestine, subsequently referred to as "vasoactive intestinal contractor (VIC)"[66]. One of the most remarkable steps in the progress of ET research, following its discovery, was the identification of isotypes of ET[67]. Analysis of human genomic sequences revealed the existence of three distinct genes for ET; these encode three distinct ET peptides and were named ET-1, ET-2, and ET-3[68].

1.11.2-Structure-Activity Relations of Endothelin Peptides

The ET family consists of the three isoforms and four highly homologous cardiotoxic peptides isolated from the venom of *Atractaspis engaddensis* , the STXs[69] . ET is a particular peptide because it is a cyclic peptide with two intramolecular disulfide bonds. All family members contain 21 amino acid residues and show complete identity at ten positions, including all four cysteine residues (positions 1,3,11, and 15), as well as at positions 8 (aspartic acid), 10 (glutamic acid), 16 (histidine), 18 (aspartic acid), 20 (isoleucine), and 21 (tryptophan)[70].

Structure activity relationship studies with the C-terminal hexapeptide of ET have been carried out to elucidate the amino acids that are important for receptor binding and agonist and antagonist activity[71]. The indole group of Trp^{21} has been found to be important for both binding and vasoconstrictor activities of ET[71]. L-stereochemistry of this residue also seems to be important[71]. Replacement of L-Trp^{21} by D-Trp^{21} markedly reduces binding and contractile activity. It has been found that sequential removal of C-terminal residues in ET decreases the vasoconstrictor potency in porcine coronary artery strips by 3 orders of magnitude. The replacement of this residue by other aromatic amino acids such as Phe or Tyr is poorly tolerated[72].

Removal of one or the other of the disulfide bridges of ET-1 reduces the potency of the peptide. The monocyclic ET-1 retaining the 1-15 disulfide bridge of ET-1 reduces the potency of the peptide[73]. The monocyclic ET-1 retaining 1-15 disulfide bridge has been found to maintain 10% of activity. The analogue retaining only 3-11 disulfide bridge has been found to be about 200-fold less active than the bicyclic ET-1[73].

1.11.3-Endothelin Genes

In the human, the ET-1, ET-2, and ET-3 genes have been mapped to chromosomes 6, 1 and 20, respectively[74]. Genetic fine mapping studies of the human

ET-1 gene has localized it to the telomeric region of chromosome 6p, close to the gene encoding the ∝-subunit of clotting factor XIII[75].

1.11.4-Endothelin Biosynthesis

The primary translation product of the human ET-1 gene is a 203 amino acid prepropeptide[84]. It is subsequently processed to the 39 amino acid prohormone big ET-1, which is secreted and circulates in plasma. Big ET-1 has 1\100 the potency of ET-1[76], but its plasma concentration is sufficiently high in conditions such as heart failure that its extracellular conversion to ET-1 could provide biologically important quantities of the latter. The endothelin-converting enzyme (ECE) then converts big ET-1 to ET-1 (Figure 6). The isolation and cloning of the ECE-1 and ECE-2 has recently been described[77]. This glycoprotein enzyme prefers ET-1 as a substrate, can be inhibited by phosphoramidon, and is a metallopeptidase. It cleaves big ET-1 between positions 21 (tryptophan) and 22 (valine) to generate ET-1[78].

Since the pressor activity of big ET-1 is not attenuated by the removal of the endothelium, the endothelium seems not to be essential for the functional conversion of big ET-1 in vascular tissues[79]. Fukuroda[80] also noted that the big ET-1-induced vasoconstriction of porcine coronary arteries, which is markedly inhibited by phosphoramidon, was not affected by the endothelium removal.

A number of reports have indicated that phosphoramidon-sensitive ECE is present as a membrane-bound form, in various organs including vascular tissues[81-82].

1.11.5-Endothelin-1

The endothelins are peptides of 21 amino acids that are produced in a wide variety of cells. ET-1 is the only family member produced in endothelial cells, and it is also produced in vascular smooth muscle cells. ET-1 is not stored in secretory granules

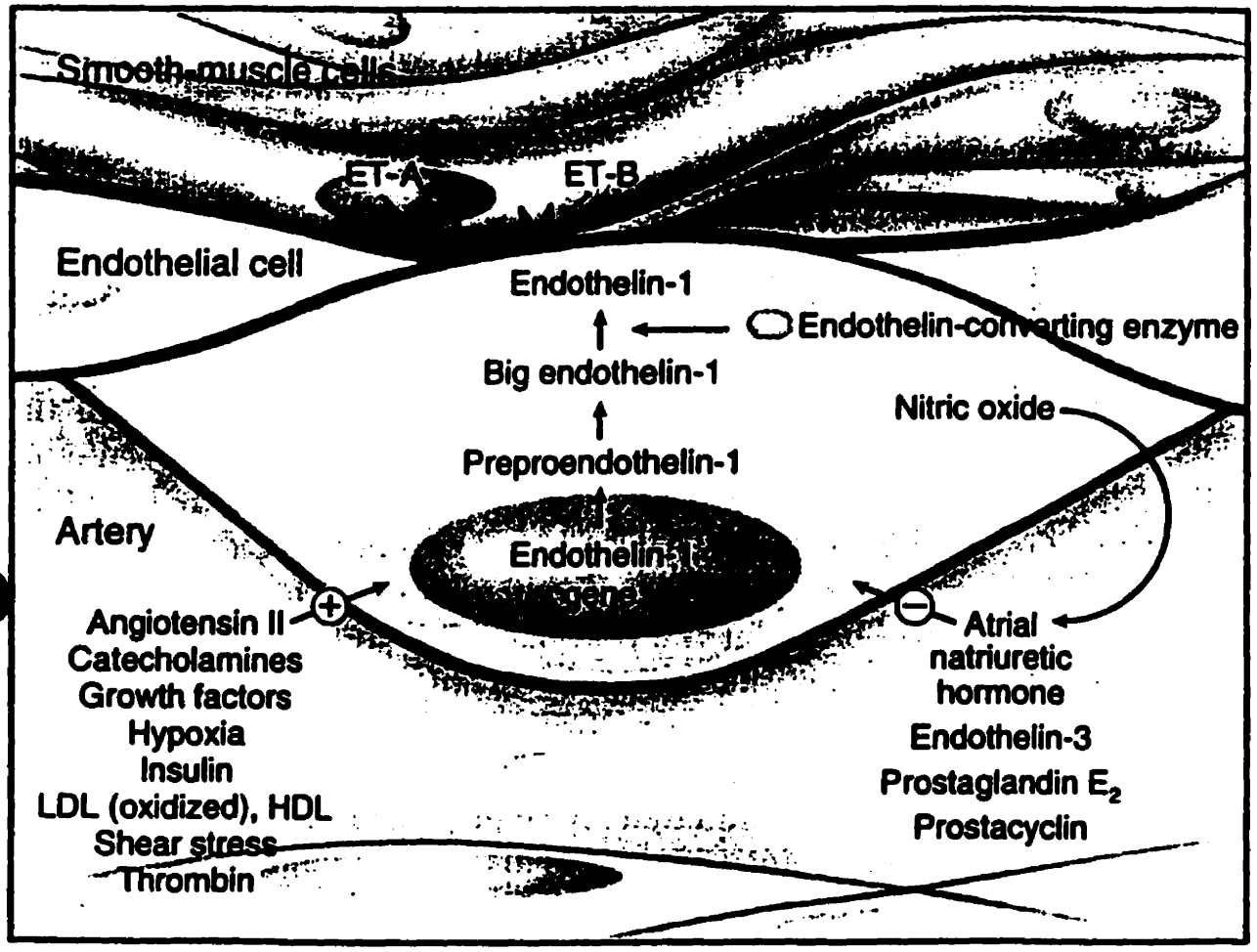

Figure 6. Regulation, processing and secretion of ET-1 in endothelial cells.

within endothelial cells[83], and important stimuli such as hypoxia, ischemia, or shear stress induce the transcription of ET-1 messenger RNA (mRNA) and the synthesis and secretion of ET-1 within 15 to 20 minutes, and the plasma half-life of ET-1 is approximately 4 to 7 minutes[85]; therefore, vascular cells can rapidly adjust ET-1 production as required for the regulation of vasomotor tone. Plasma ET-1 is cleared mostly (80 to 90 percent) by the lungs during first passage[85].

As much as 75 percent of ET-1 secretion from cultured endothelial cells is toward the vascular smooth-muscle (abluminal) side of the cells[86], where it can bind to specific receptors on the muscle cells and cause vasoconstriction. It is unlikely that this pool contributes to the ET-1 concentration in plasma. Therefore, ET-1 should be regarded more as a paracrine than as an endocrine hormone. Plasma ET-1 measurements are nevertheless useful, because plasma concentrations have been found to correlate well with the severity of disease, such as congestive heart failure[87], and may have prognostic or diagnostic value. ET-1 is also produced by neurons and astrocytes in the central nervous system, endometrial cells, hepatocytes, kidney mesangial cells, Sertoli cells, and breast epithelial cells[8,69].

1.11.6-Endothelin-2

ET-2 is produced predominantly within the kidney and intestine, with smaller amounts produced in the myocardium, placenta, and uterus, but the cells of origin are not clear. ET-2 has no unique physiologic functions, as compared with ET-1.

1.11.7-Endothelin-3

Like ET-1, ET-3 circulates in the plasma, but its source is not known. ET-3 has been found in high concentrations in the brain[89] and may regulate important functions in neurons and astrocytes, such as proliferation and development. It also is found throughout the gastrointestinal tract and in the lung and kidney.

1.11.8-ET Receptors: ET_a receptor

ET_a receptors have 10 times greater binding affinity for ET-1 than ET-3[90] and are expressed abundantly on vascular smooth-muscle cells and cardiac myocytes. These receptors mediate the vasoconstrictor action of ET-1, although Et_b receptors may contribute to this action in some vascular beds. The vasoconstriction is related to the ability of ET_a-activated receptors to stimulate phospholipase C, which leads to the formation of inositol 1,4,5-triphosphate and diacylglycerol (Figure.7). The former increases the intracellular calcium concentration, which in turn causes the vasoconstriction[91]. The vasoconstriction persists after ET-1 is removed from the receptor[92], probably because the intracellular calcium concentration remains elevated. Nitric oxide shortens the duration of vasoconstriction by accelerating the return of intracellular calcium to its basal concentration[93]. Diacylglycerol and calcium stimulate protein kinase C which mediates the mitogenic action of ET-1[94].

Knockout of the gene encoding the ET_a receptor causes severe craniofacial and thoracic blood vessel malformations, which suggests that it is important in the normal development of the pharyngeal arches, heart and great vessels[95].

1.11.9-ET_b Receptors

ET_b receptors are expressed predominantly on endothelial cells[96] and to a much smaller extent on vascular smooth-muscle cells. ET_b receptors bind to all three ET isoforms with similar affinity, and mediate the actions of either peptide. The effects of the activation of ET_b receptors (on vascular smooth muscle cells) are similar to those of the activation of the ET_a receptors in stimulating the activation of phospholipase C, the generation of inositol 1,4,5-triphosphate and diacylglycerol, and the mobilization of calcium. ET_b receptors are also found on endothelial cells where they mediate

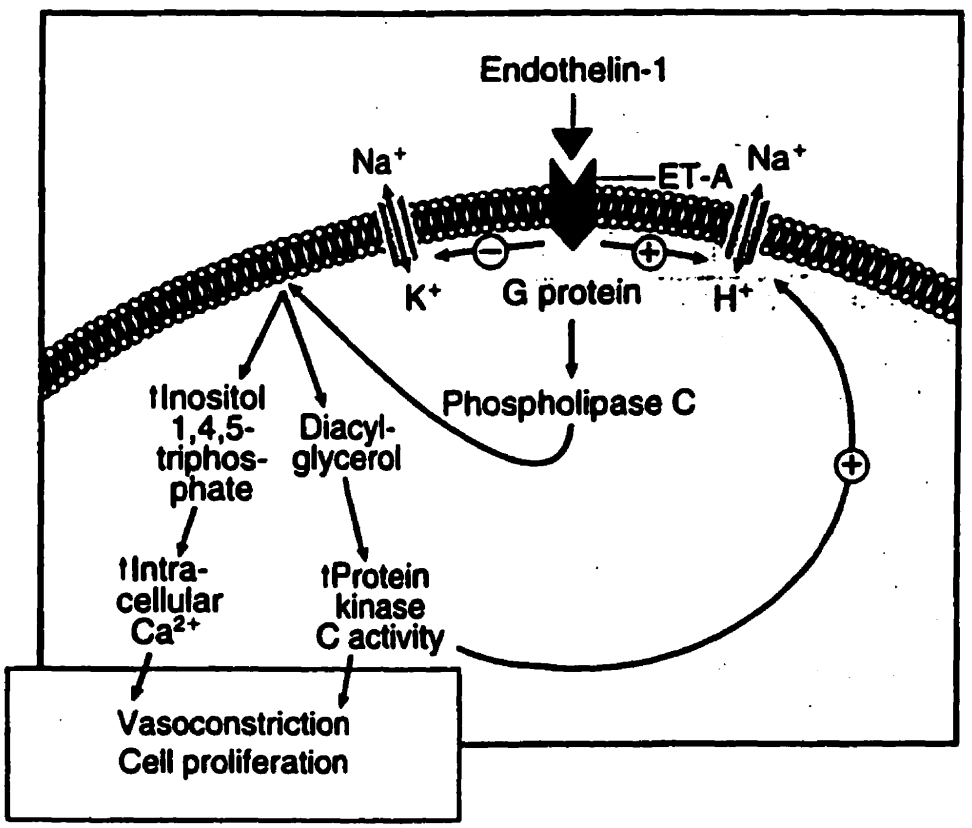

Figure 7. Mechanisms of signaling induced by ET-1 through the ET_a receptor.

vasorelaxation. The vasodilation is probably caused by increased production of nitric oxide and prostacyclin [97,98]and the activation of potassium channels.

The activation of ET_b by ET-3 is critical for the normal development of cells derived from neural-crest precursors[99]. Epidermal melanocytes and ganglionic neurons in the intestine do not develop when the ET_b receptor functions improperly or when ET-3 is not produced[99]. Mice bearing inactivating mutations of the gene for the ET_b receptor have aganglionic megacolon and extensive white spotting of their coats[100].

1.11.10-Regulation and Expression of Endothelin Receptors

The regulation of the production of the ET receptors oftens parallels that of the ETs. For example, hypoxia rapidly stimulates the productiion of ET-1 and ET_a receptors in endothelial cells and vascular smooth muscle cells, respectively[88].

The differences in tissue-specific expression of the two receptors contribute to the different actions of the three ETs. Within a particular tissue, the distribution of ET_a and ET_b receptors also varies. In the kidney, ET_a receptors are prominent in the vasa recta and arcuate arteries, whereas ET_b are found predominantly in the collecting ducts, suggesting different roles in the modulation of salt and water reabsorption.

The regulation of ET receptors is similar in penile tissue. There seems to be a close association between vascular ET-1 binding sites and functional effects of ET-1. For example, in diabetic rat corpus cavernosum tissue, both ET-1 and the ET_a receptor increase[160].

1.11.11-ET_a and ET_b receptor antagonists

Endothelin antagonists are able to inhibit endothelin-1. Endothelin antagonists are important tools used to elucidate the effects of endothelins on different blood vessels. Specific ET antagonists have determined that the human microcirculation of the skin activates mainly Et_a receptors[101]. Furthermore, endothelin antagonists can help to

characterize the distribution of the endothelin receptors and help to detect new receptor subtypes.

The role of endothelin in disease is not yet fully elucidated. Although in different pathological cardiovascular situations such as atherosclerosis, myocardial infarction, coronary spasm, pulmonary and possibly arterial hypertension, endothelin plasma levels have been found to be elevated[102-106] (Figure.8), the pathophysiological significance of these findings remain unclear.

1.11.12-Pharmacological Interactions of Endothelin with Various Regulatory Agents

The direct action of ET via activation of its receptors, and its interactions with various regulatory agents, all occuring simultaneously, governs the final response of ET. During the past few years, investigators have shown that these interactions are involved in a variety of physiological functions.

1.11.13.1-Nitric Oxide (NO) and ET interactions

Under normal physiological conditions, there exists a delicate balance between endothelium-derived relaxing factors and endothelium-derived contracting factors. The endothelial cell has a unique intrinsic feature; it produces the most potent vasoconstrictor peptide (ET-1), and yet it also releases an equally potent vasodilator substance, NO. ET is known to cause an initial and transient vasodilation followed by a sustained vasoconstriction. The vasodilation is thought to be via the activation of ET_b receptor which causes release of vasodilators, one of which is NO. It has been shown that acetylcholine[107-109] and bradykinin[110] antagonize the contractile effects of ET by receptor mediated release of EDRF. In contrast to arteries, the effect of ET is more

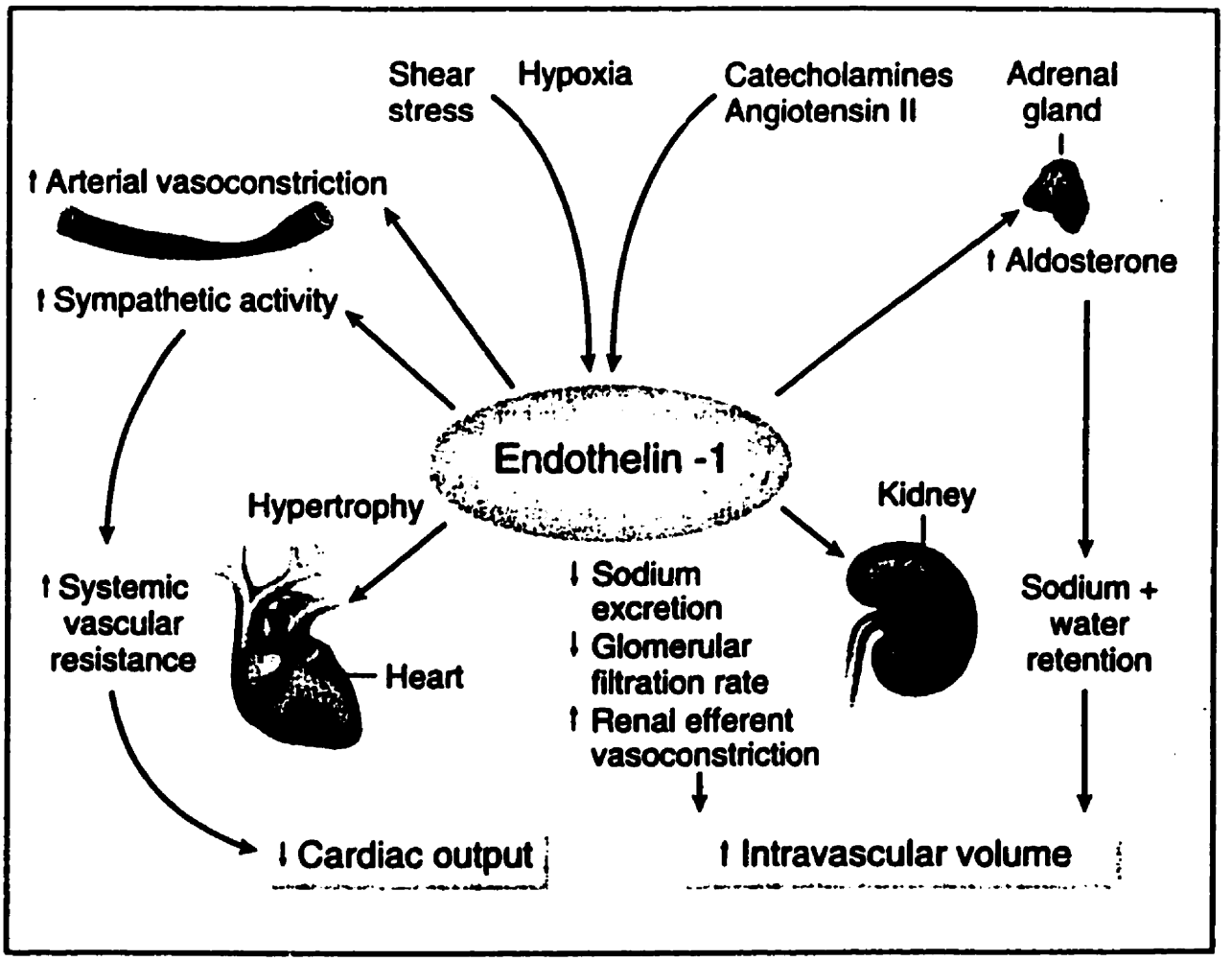

Figure 8. Suggested roles of ET-1 in different pathological cardiovascular situations.

pronounced in the veins because in veins the potency of NO to antagonize ET-induced contractions is less marked[107,108,112].

NO has also been reported to modulate the actions of ET. NO suppresses the release of ET from endothelial cells[113] and it mediates the antiaggregatory action of ET on platelets[114]. Cyclic GMP, a second messenger for NO, can interfere with the signal transduction pathway of ET[115]. Therefore, NO appears to be one of the physiological modulators of ET[117] which can prevent excessive vasoconstriction and thrombic occlusion of the vascular bed. Impaired production of NO, as in hypertension, irradiation or atherosclerosis could result in an unopposed release of ET and pathological constriction of diseased blood vessels.

1.11.13.2- Noradrenaline effects on ET

Catecholamines can stimulate ET gene expression in vascular smooth muscle cells[64]. Cultured porcine endothelial cells derived from the aorta, spontaneously released immunoreactive ET which could be potentiated by adrenaline via α-adrenoceptors[118].

Since neural release of noradrenaline (NE) is regulated by presynaptic α_2-adrenoceptors and adenosine receptors, it is possible that ET interacts with these regulatory mechanisms or acts directly on some pathways of NE release. Centrally administered ET-1 has been shown to elicit a pressor response by activating the sympathetic nervous system[119-120]. In addition to the effect on neural release, ET also modulates the catecholamine release from other storage sites. For example, ET-1 has been shown to enhance the efflux of adrenaline and noradrenaline from adrenal chromaffin cells.[121]

1.11.13.3-Thrombin effects on ET

Thrombin exhibits a variety of interactions with ET. The expression of the ET gene is stimulated by thrombin in endothelial cells[122] and mesangial cells[123]. It releases ET-1 and Big-ET-1 from aortic strips with endothelium[124](as shown in a porcine model) ET-1 from isolated glomeruli and mesangial cells[125] (as shown in a rat animal model), tracheal epithelial cells[126] (as shown in a rabbit animal model) and astrocytes[127] by a mechanism that probably involves intracellular calcium mobilization and activation of PKC[128].

Since thrombin is generated during clotting of blood, an increased production and release of ET-1 from endothelial cells may be of importance in the pathogenesis of vasospasm associated with activation of the coagulation cascade. Activation of the coagulation cascade with concomitant formation of thrombin is a known event in unstable angina and myocardial infarction. Thrombin stimulates platelet aggregation, whereas ET inhibits it[129,130]. ET-1 inhibits the release of plasminogen activator inhibitor (PAI-1) antigen[131],and has a suppresive effect on thrombin-stimulated release of both tissue plasminogen activator (t-PA) and plasminogen activator inhibitor (PAI-1) from endothelial cells[132]. This suggests that ET-1 has as antifibrinolytic action on thrombin-induced fibrinolysis.

1.11.13.4-Calcitonin-Gene Related PolyPeptide (CGRP) effects on ET

CGRP is a potent vasodilator originating from sensory nerve fibers. CGRP has also been shown to colocalize with ET-1 in several tissues. Co-infusion of ET and CGRP in rats inhibits the ET-induced contraction[133]. CGRP decreases ET-1 induced contractions and ET-1 decreases CGRP-induced relaxations[134]. This interaction can be of pathophysiological significance as increased levels of CGRP[135] and ET [136]have been reported in subarachnoid hemmorrhage.

1.11.13.5-Endothelin And Renin-Angiotensin System

The renin-angiotensin system acts as a principal regulator of the extracellular fluid volume. ET-1 has actions similar to angiotensin-II (A-II), in that it can also cause a net reabsorption of Na^+ from the proximal tubules. ET-1 and A-II exhibit a synergistic effect on the systolic pressure when given at subthreshold doses[137]. A-II stimulates ET gene expression in endothelial cells[138-140], vascular smooth muscle cells (VSMC)[141-142] and cardiomyocytes[143]. It also increases the production of ET-1 in human endothelial[144], VSMC[141] and mesangial cells[144].

Systemic infusions of ET-1 dramatically elevate the plasma renin activity as well as aldosterone levels[144-145]. The increase in aldosterone can be indirect via renin activation, or due to direct action of ET-1 on the adrenal glomerulosa cells[146]. It was also shown that ET-1 exerts a direct stimulation of aldosterone secretion[147] in a Ca^{+2} -dependent manner, and it potentiates A-II mediated aldosterone stimulation through a mechanism involving PKC[148]. ET-1 can also activate the vascular renin-angiotensin system which might lead to an increase in plasma levels of renin[149].

1.11.14-Mitogenic Interactions Of Endothelin

In addition to being a potent vasoconstrictor, ET also serves as a growth promoting factor for many cell types. It is known to stimulate c-fos and c-myc expression and proliferation of vascular smooth muscle cells in a dose-dependent manner. It also influences DNA synthesis, expression of protooncogenes, cell proliferation and causes hypertrophy. The mitogenic potency of ETs is low as compared to other known mitogens, but together, they can exert significant mitogenic actions in a variety of cell types[69].

Within the vessels of the penis, mitogens such as platelet-derived growth factor and transforming growth factor are known to cause migration and proliferation of vascular smooth muscle cells. ET-1 can act as a comitogen with these growth factors

and together could be in part responsible for the atherosclerosis seen which results in a gradual loss of erection seen in patients who undergo radiation therapy for prostate cancer.

1.11.15-ET and Penile erection

During erection, the resistance to penile blood flow is low due to active dilatation of penile arteries and sinusoids. For detumescence to occur, the smooth muscle of penile arteries and erectile tissue needs to contract. This contraction is probably mediated mainly by the release of noradrenaline acting on post-junctional α adrenoceptors. However, additional mechanisms besides release of noradrenaline are likely to be involved in the long-term maintenance of the high penile smooth muscle tone necessary for keeping the penis in the flacid state. Endothelin-1 may well be such a factor. Since the discovery of ET-1, greater insight into corporal physiology has been elucidated. The demonstration that cultured endothelial cells from human corpus cavernosum are expressing ET mRNA, the presence of specific binding sites for ET-1 on human corporal smooth muscle cells, the effect of ET-1 on intracellular Ca^{2+} levels and, particularly, the long lasting and potent contractile effects of ET-1 on human corporal smooth muscle strips strongly suggest that ET-1 may play a pivotal role in the regulation of tone in the penis[150].

Experiments have shown that ET-1 potently and concentration-dependently contracts isolated human and rabbit corpus cavernosum[150,152]. The threshold concentration of the peptide is significantly lower in human corpus cavernosum than in rabbit corpus cavernosum. Furthermore, it was shown that the presence of the receptors were distributed uniformly within the cavernous tissue. Numerous binding sites were observed on the smooth muscle cells of the deep penile artery. Extracellular Ca^{2+} was found to generally be a prerequisite for the major part of the ET-1-induced contraction[151]. ET-1 may play a role as a modulator of other agents that are believed to

be of importance in the regulation of penile smooth muscle tone. For instance, it has been reported that ET-1 increases the release of various vasoactive substances, including thromboxane A_2, prostacyclin and nitric oxide[153]. Furthermore, several investigations show that ET-1 has prejunctional inhibitory effects on the release of noradrenaline and acetylcholine[154]. Contractions were evoked in human corpus cavernosus tissue also by ET-2 and ET-3, although these peptides had a lower potency than ET-1[150].

Penile veins have also been shown to be sensitive to ET-1. Compared to the corpus cavernosum, ET-1 elicited contraction at a significantly lower threshold concentration in the penile cicumflex vein. Futhermore, the effect of ET-1 in veins seems to be independent of influx of extracellular Ca^{2+}. It has been suggested that ET-1 may play a role in the veno-occlusive mechanism[150]. When the sinusoids are filled with blood during tumescence and erection, the subtunical veins are compressed against the tunica albuginea. Thus, venous outflow is impaired. It has been shown that an increase in flow rate over aortic endothelial cells leads to an instant and reproducible release of ET-1, which is sustained during the whole period of high flow[157]. If true also for the corpus cavernosum, the post-cavernosal venules and emissary veins would be exposed to blood with a relatively high ET-1 concentration during tumescence. Provided that the effects of ET-1 in these veins are similar, this model would represent an active, non-neuronal veno-occlusive mechanism that could participate in the induction and maintenance of erection.

In conclusion, the expression of ET-1 mRNA in cultured endothelial cells from corporal tissue, the presence of specific and functional ET-1 receptors , and the physiological role given to ET-1 in vitro make it reasonable to believe that ET-1 may play a role in the regulation of penile smooth muscle tone in vivo.

1.11.16-ET-1 and RAI

Endothelial cells are susceptible to damage. Any insult given to these cells could result in a pathological condition. Radiation is a known insult able to inflict damage to the endothelial cells. As a result, the level of vasoactive substances produced by these cells can be altered. Consequently, the levels of ET-1 could be altered, upsetting the normal balance of vasoactive substances, leading to abnormal vascular tone. This assumption comes from several experiments showing abnormal levels of ET-1. For instance, experiments have shown a dose-dependent increase production of ET-1 by human keratinocytes irradiated with ultraviolet B radiation[158].

RAI is a frequently encountered side-effect of radiation therapy for prostate cancer. Furthermore, in vitro experiments have shown that the etiology of many patients with erectile dysfunction is due to an increased contractility of the vessels that supply the penis. There is experimental evidence that ET increased contractile effects can be involved in erectile dysfunction. Extrapolating to RAI, perhaps there is an increased ET-1 production following radiotherapy. The source of the ET could be the pelvic vasculature endothelial smooth muscle cells and/or the prostate. The prostate can be a major source of ET production, since it has been estimated that the prostate produces 0.58pg/mg tissue[159]. Therefore, high levels of ET released into the local circulation could be seen following irradiation-induced destruction of the prostatic capsule. As a result, an increased ET-1 production released from the pelvic vasculature endothelial or smooth muscle cells and/or the prostate might act as a paracrine/autocrine factor that could alter tissue contractility by three proposed mechanisms:

1. ET-1 could achieve the effective local concentrations, required for activation of the putative ET_A receptor subtype and thus elicit a direct vasoconstrictor effect on the smooth muscle cells.

2. At lower concentrations, ET-1 can potentiate the actions of other vasoactive agents or neurotransmitters.

3. Perhaps the ET_b receptor found on endothelial cells could be deficient leaving only its vasoconstricting effect on the smooth muscle cells.

ET-1 and NO are two vasoactive substances produced by endothelial cells and are known to have opposing effects on vascular tone . Previous investigators showed a dose-dependent decrease in NOS-containing nerve fibers in the rat, given irradiation to the prostate. If ET-1 acts to antagonize NO, then perhaps ET-1 may increase in response to irradiation given to the prostate. The increase in ET-1, in the long-term, could be responsible for the atherogenic effects seen in patients.

1.12 Specific Research Aims

The primary objective of the work presented in this thesis was to further elucidate the role of endothelin in radiation-associated impotence. This study can however be subdivided into two parts:

(1). In the first part of the study, we assessed whether endothelin levels change after radiation to the prostate in a rat animal model. A time course of ET-1 rise at a low dose (1000 cGy) and a high dose (2000 cGy) was determined.

(2). We evaluated whether penile erection in a rat model be potentiated by an ET_a receptor antagonist after radiation to the prostate.

MATERIALS AND METHODS

2.1 Study population

Ninety Sprague-Dawley rats received radiation to the pelvic region . For the radioimmunoassays, rats were divided into three groups: control (no irradiation) (n=20), 1000 cGy (n=20), and 2000 cGy (n=20). Rats for the in vivo study were divided into two study groups: control (n=10) and 2000 cGy (n=20).

2.2 Time Points

For the radioimmunoassay, rats were evaluated at the following time points after irradiation: 6 hours, 1 day, 2 days, 5 days, 1 week, 10 days, 20 days, 1 month, and 2 months.

For the in vivo study, rats were evaluated at three time points: 20 days, 1 month, and 2 months.

2.3 Radiation protocol

Animals were anesthetized using pentobarbitol (45 mg/kg-i.p.). Radiation was delivered in the department of Radiation Oncology at the Jewish General Hospital. The irradiation procedure was performed by the radiotherapy technicians, under the supervision of Dr. G. Shenouda. Each rat was immobilized in the radiation field. Radiation was delivered to the prostatic region by a 4.5 x 4.5 cm field in a single fraction. Special attention was given to protect both testis. A previous study showed that the levels of FSH and LH rise after radiation therapy indicating testicular injury which could affect the results[16]. Protection was assured by using a lead shield over the testis. Treatment was provided with 250-kVp X rays at a dose rate of 424 cGy/min. Animals were then evaluated at their specific time points.

2.4 Evaluation of the effects of irradiation

2.4.1.1 Histopathological evaluation

Histological analysis was done to confirm radiation-induced damage to the prostate. Rat prostatic tissue was fixed in 10% buffered formaldehyde. Tissues were step-sectioned at 3 mm intervals and paraffin-embedded in toto.

Histological analysis was done to confirm no testicular damage, as outlined in section 2.3. Testes were fixed in 10% buffered formaldehyde. Each testis was step-sectioned at 3 mm intervals. A whole mount section along the longitudinal axis was paraffin-embedded. Histological sections were stained with hematoxylin-eosin. Testes at both dosages and all time points were evaluated. Gross and histological examinations were performed without knowledge of the irradiation status (using a code number).

2.4.1.1 Determination of Testosterone Levels

Serum samples were assessed for total testosterone using a radioimmunoassay kit by the Department of Biochemistry at the Jewish General Hospital.

2.4.2 Endothelin-like immunoreactivity content

2.4.2.1 Plasma content

2.4.2.1.1 Blood collection

i) Before irradiation

Under anesthesia, 0.5 ml of blood was drawn from the tail artery 3 days before irradiation. Blood samples were collected in tubes containing an anticoagulant (3.5% sodium citrate), centrifuged at 15 000 g for 1 minute and stored at -80°C. The supernatant samples were taken through Sep-Pak cartridges (Waters). Cartridges were activated with methanol (100%) and 1% trifluoracetic acid(TFA) followed by 0.1% TFA (3 x1ml). The absorbed fraction was eluted with 100% methanol and the eluate

was dried with the use of a speed vacuum. The dessicated residue was reconstituted in an appropriate volume of assay buffer, and frozen at -20°C until evaluation of immunoreactivity content.

ii). After irradiation

After completion of the in vivo physiological assessment , 1 ml of blood was taken from the carotid artery. The same procedure was followed as above (section 2.4.2.1.1.i).

2.4.2.2 Tissue content

2.4.2.2.1 Peptide extraction

In order to determine endothelin levels in tissue, the following extraction procedure was followed. Tissues were placed directly into 200 ul of hot (60°C) extraction buffer in a 1.5 polypropylene microcentrifuge tube and then immediately boiled (100°C) for 10 minutes. An extraction buffer was used which consisted of: 2 M acetic acid, 11.4 mM HCl, 1 mM disodium ethylenediamine tetraacetate (EDTA), and 1 mM dithiothreitol, and several protease inhibitors: 1mM 4-(2-aminoethyl)-benzesulfonyl fluoride-HCl (AEBSF) (Calbiochem), 2 ug/ml aprotinin (Calbiochem), 100 uM leupeptin (Calbiochem), 1 ug/ml cystanin (Calbiochem), and 1 mM benzamidine (Sigma). After boiling, all samples were cooled on ice before homogenization for 2 minutes using a polytron (Brinkman). Subsequently, the samples were centrifuged at 3835 g at 4°C for 15 minutes. The supernatant was then subjected to lyophylisation. The luophylised powder was then transferred to a fresh tube and this stored at -80°C until required.

2.4.2.3 Radioimmunoassay (RIA) Procedure

Endothelin concentrations were measured by radioimmunoassay. A commercially available ET-1 kit (Phoenix, CA) was used for the RIA, with an antibody which recognizes ET-1 at 100%, ET-2 and ET-3 at 7%, and human Big-ET-1 at 17%.

The antibody supplied in the kit is a rabbit polyclonal antiserum. In addition, ^{125}I-labelled ET-1 peptide used as the competitive marker. The samples were reconstituted in the supplied RIA buffer before the rabbit anti-ET-1 sera addition followed by an overnight incubation at 4°C. The following day, ^{125}I-ET-1 was added; the samples were vortexed and incubated at 4°C overnight. On day 3, goat anti-rabbit immunoglobulin G serum and normal rabbit serum were added and the mixture was incubated at room temperature for 120 minutes. Additional RIA buffer was added, and tubes were centrifuged at 3000 rpm for 20 minutes. The supernatant was delicately aspirated off avoiding dislodging the pellet, which was then subjected to radioactive quantification in a γ-counter. ET-1 protein levels were then determined by plotting the value of the unknowns on the standard curve.

2.4.4. In Vivo Physiological Assessment

2.4.4.1. Physiological Assessment

Rats were anesthetized using sodium pentobarbitol (45 mg/kg-i.p.). The right carotid artery was catheterized with PE-50 tubing for monitoring the systemic blood pressure. Through a lower midline abdominal incision, the lateral prostate space was dissected and the MPG identified. The cavernous nerve has a constant course running from the MPG on the dorsolateral prostate surface vertically covered by a thin filmy semitransparent fascia. This is easily apparent using optical magnifications (X10 to X

40) provided by a Zeiss SR stereo operating microscope. The cavernous nerve was freed from its fascial attachments and hooked using a bipolar steel electrode 3 to 4 mm distal to the MPG. Through a perineal-scrotal incision, the right penile crus was exposed by spreading the overlying ischiocavernous muscle. A 23G needle filled with a dilute heparin solution (50 U/mL) and connected to PE-50 tubing was inserted into the penile crus. Systemic pressure and maximum intracavernous pressure (ICP) were measured and recorded using Labview 2 program software (National Instruments, Texas). Electrostimulation was carried out with a delicate stainless steel bipolar hook electrode (Figure 9.). The two poles of the electrode were seperated by 2 mm with each pole 0.2 mm in diameter. Monophasic rectangular pulses were delivered from the module at a pulse width of 0.2 ms, frequency of 20 pulses per second, and current of 2 mA. The duration of stimulation was 40 seconds. Each animal was stimulated twice before and after injection of the ET_A receptor antagonist (BQ-123). The resting period between stimulations was 10 minutes. We have shown previously that 10 minutes rest provides reproducible results.

2.4.4.2. BQ-123 Administration

The specific ET_A receptor antagonist, BQ-123 (American Peptide, CA) was used to block the actions of endothelin after irradiation. The left penile crus was exposed by spreading the overlying ischiocavernous muscle. A 30G needle connected to PE-20 tubing was inserted into the left crus to serve as the medication line. 0.5 mg of BQ-123 was dissolved in 100 ul of DMSO, and diluted in 600 ul of HPLC water. 30 ul of the drug was inserted into the penis. Sham-irradiated rats underwent the same procedure with saline rather than the antagonist.

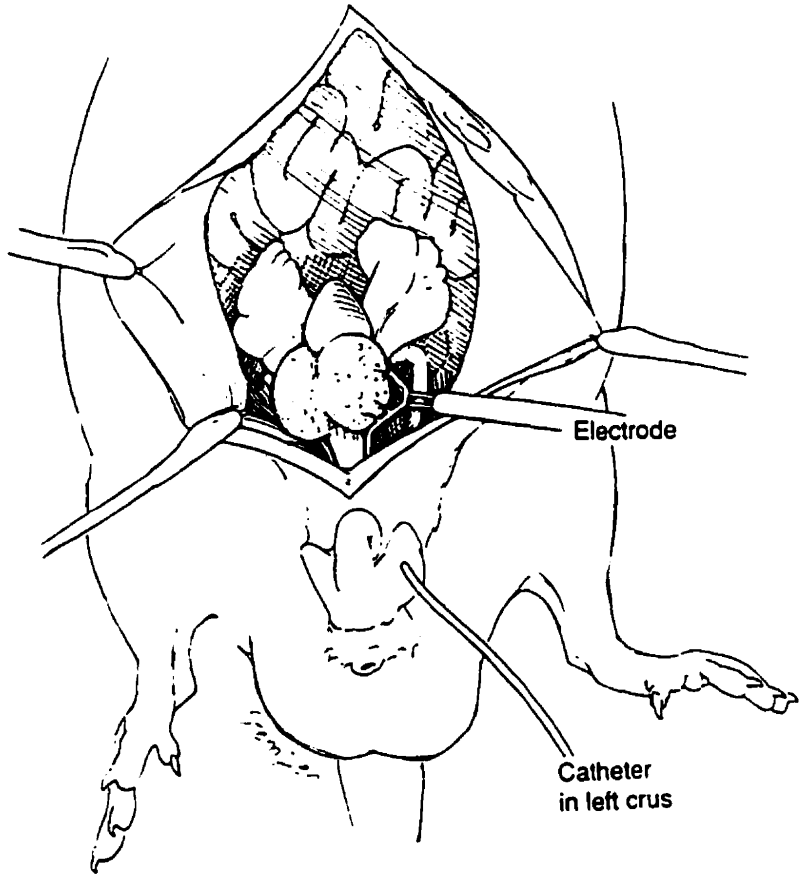

Figure 9. Schematic drawing depicting the setup of the physiological experiment done on the rat animal model.

2.5 Statistical analysis

The results are expressed as means ± standard error of the mean. Differences in between groups were compared using a one-way analysis of variance (ANOVA), and P values ≤ 0.05 were considered to be statistically significant.

RESULTS

3.1 Morphological assessment of testes following radiation

Gross and histological examination were performed in order to determine if the shielding of the testes was effective. The following observations were made:

3.11 1000 cGy, 1 day

Both testes were similar, each one measuring 2.0 x 1.1 cm. No specific pathological alteration was revealed on microscpoic examination.

3.12 1000 cGy, 10 days

One testes measured 2.1 x 1.1 x 1.1 cm, whereas the controlateral testis measured 2.0 x 1.1 x 1.0 cm. The testes were grossly normal. Only rare tubules show disorganization and sloughing of the germ cell population; such finding is non-specific, although occasionally associated with some "obstruction" in the human testis. Otherwise, the microscpoic apperance is within normal limits.

3.13 1000 cGy, 20 days

Both testes are similar, each one measuring 2.0 x 1.1 x 1.1 cm. Both testes were grossly normal. No specific alteration on microscpoic examination.

3.14 1000 cGy, 1 month

Both testes were similar, each one measuring 2.1 x 1.1 x 1.1 cm. Both testes were normal. Except for rare burn-out peripheral seminiferous tubules (with dystrophic calcication), there is no specific pathologic alteration on microscopic examination.

3.15 2000 cGy, 1 day

Both testes are similar, and grossly normal. Each onr measures 2.0 x 1.1 x 1.1 cm. No specific pathological alteration on microscopic examination.

3.16 2000 cGy, 10 days

Both testes are similar, each one measuring 2.1 x 1.1 x 1.1 cm. Both testes are grossly normal. No specific pathological alteration on microscopic examination, apart from rare tubules with some disorganization and sloughing of the germ cell population.

3.17 2000 cGy, 20 days

Both testes were similar, each one measuring 2.1 x 1.1 x 1.1 cm. Both testes were grossly normal. Except for rare burn-out peripheral seminiferous tubules (with dystrophic calciferation), there is no specific pathologic alteration on mimicroscopic examination.

3.18 2000 cGy, 1 month

One testis measures 2.0 x 1.1 x 1.1 cm, whereas the contralateral testis measures 1.7 x 1.0 x 1.0 cm. The larger testis is grossly normal. Regarding the smaller testis, about 1/3 of the cut surface (at one pole) appears slightly paler. The larger testis is within normal limits on microscopic examination. The smaller testis shows a regional pattern (25-30% surface area) characterized by a loss of germ cell population (presence of Sertoli cells only) within seminiferous tubules. Otherwise, the residual seminiferous tubules show a range of relatively well preserved to moderately decreased germ cell population with otherwise evidence of maturation.

In conclusion, only one testes (2000 cGy, 1 month) showed any significant pathological alteration consistent with radiation0induced injury.

3.2 Histological assessment of rat prostate following radiation

A rat prostate was observed histologically, two months following radiation, to confirm radiation-associated damage. The following observations were observed: Approximately 65-70% of the prostatic glands were atrophied, being characterized by a loss of intraglandular infolding/ festooning (which is observed in normal growth), a low globular basophilic material or debris of undetermined nature). The non-atrophic glands appear with a somewhat regional pattern of distribution, including the most peripheral aspect of the prostate. The lining epithelium of latter glands shows a significant degree of radiation-related atypia, including: abnormal cell polarity/ stratification, nucleomegaly (nuclear enlargement) with hyperchromasia and distinctive nucleoli, binucleation (ie. syncytial forms), increased volume of cytoplasm, and occassional cells with cytoplasmic and/ or nuclear vacuolar changes. Furthermore, within these glands, evidence of apoptosis (significant number of apaptotic cells) and mitosis (rare mitosis) is observed. Glandular intermediate forms (on their way to become atrophic) are also observed. The intervening stroma is somewhat edematous with focal mixed cell inflammation (lymphocytes, plasma cells and polymorphonuclear neutrophils). Thus, in conclusion, the rat prostate was consistent with radiation-induced glandular damage, including focal near-end stage glandular atrophy.

3.3 Testosterone levels

Testosterone levels were measured in the serum to evaluate whether radiation altered the level of this hormone. Our studies indicate that erectile dysfunction induced by radiation damage had no effect on serum testosterone levels.

Table 2. Testosterone levels at specific time points after radiotherapy.

	Testosterone levels (pg/g tissue)
6 hours	17.7
1 day	17.7
2 days	17.5
5 days	17.3
1 week	17.1
10 days	17.1
20 days	17.2
1 month	16.9
2 months	16.9

3.4 Plasma levels of ET-1

In order to determine if ET-1 levels increased in the blood following radiation, a time course was performed. Our results show no significant increased in the 1000 cGy group (2 fmol ET-1/tube±0.7) compared to the control group (1.5 fmol ET-1/tube±0.9). However, in the 2000 cGy group, a significant elevation was observed only starting at the tenth day (5fmol ET-1/tube) (see Figure 13).

3.5 Tissue levels of ET-1

Tissue levels for ET-1 were assessed in prostatic and penile tissues. In the penile tissue, both the control group and the 1000 cGy group had the same levels of ET-1 (1.2pg/g tissue±0.9). However, in the 2000 cGy group, at 20 days and at 1 month, there

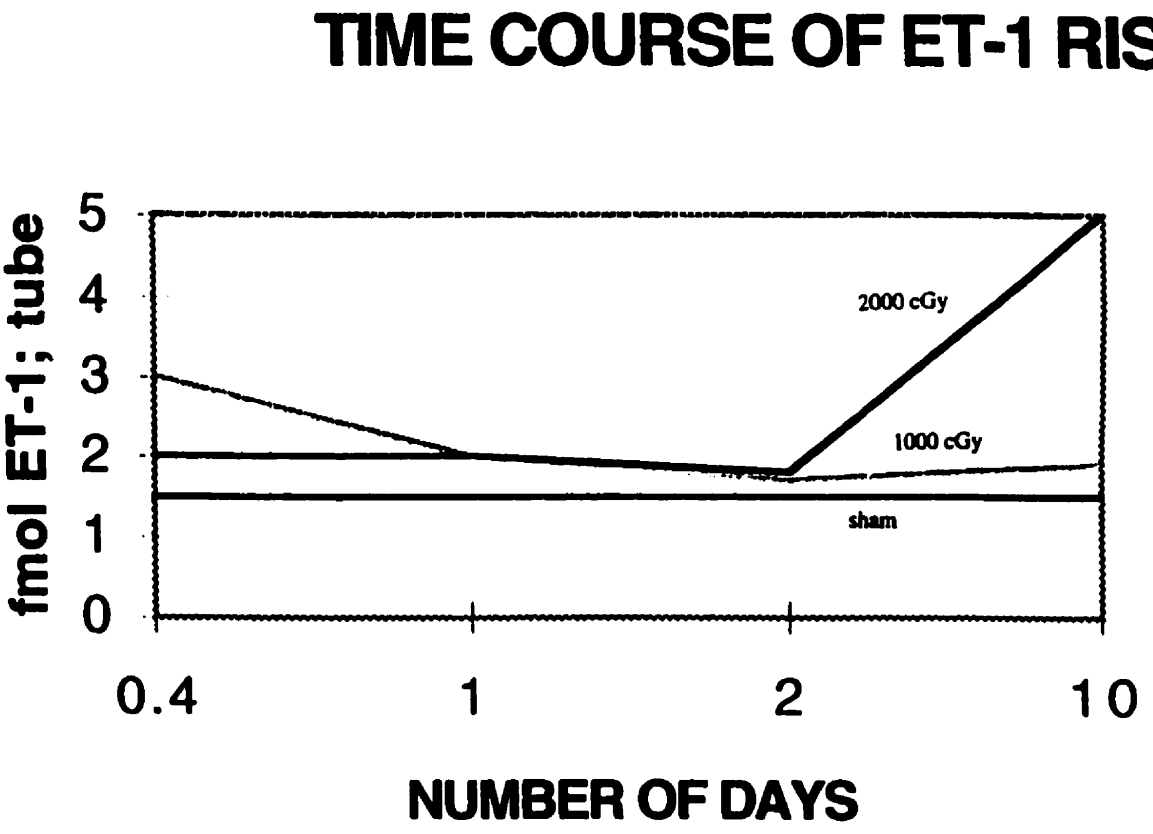

Figure 10 . Time course of ET-1 rise in plasma of the rat.

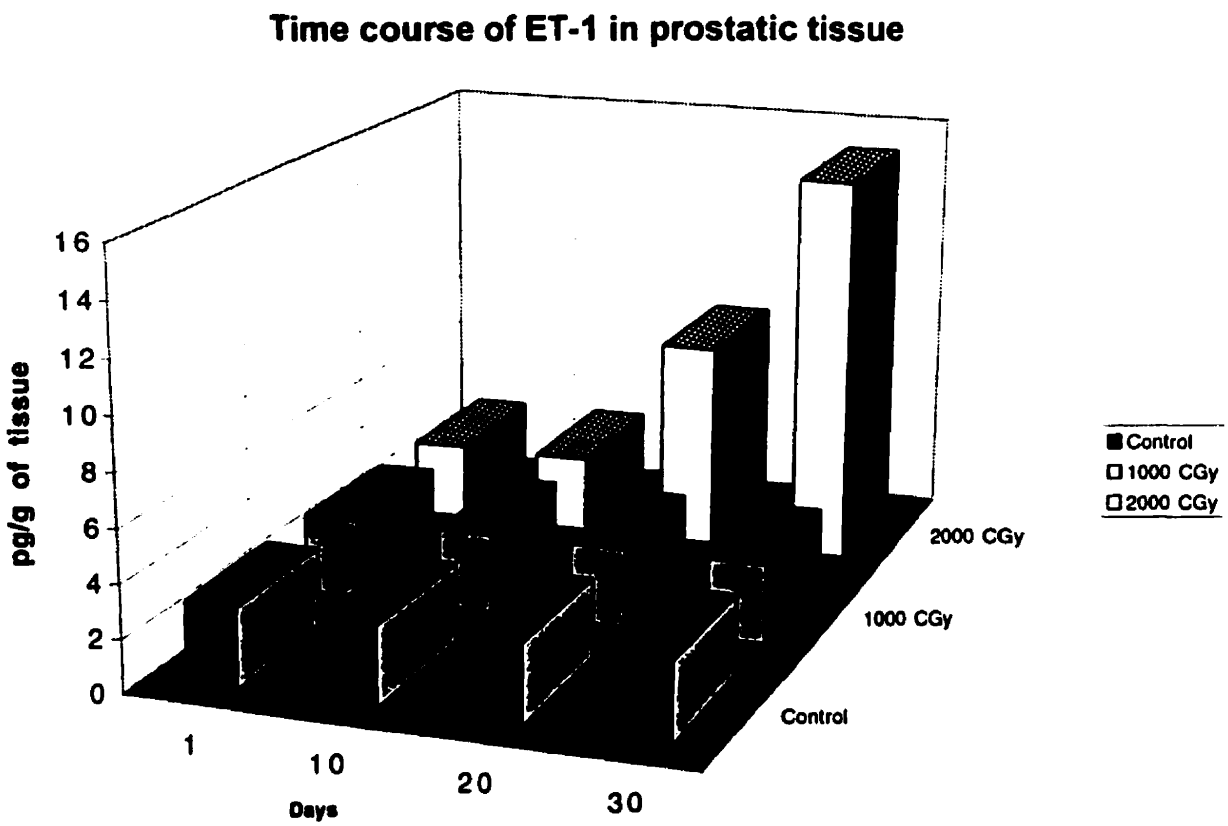

Figure 11 . Time course of ET-1 rise in prostatic tissue.

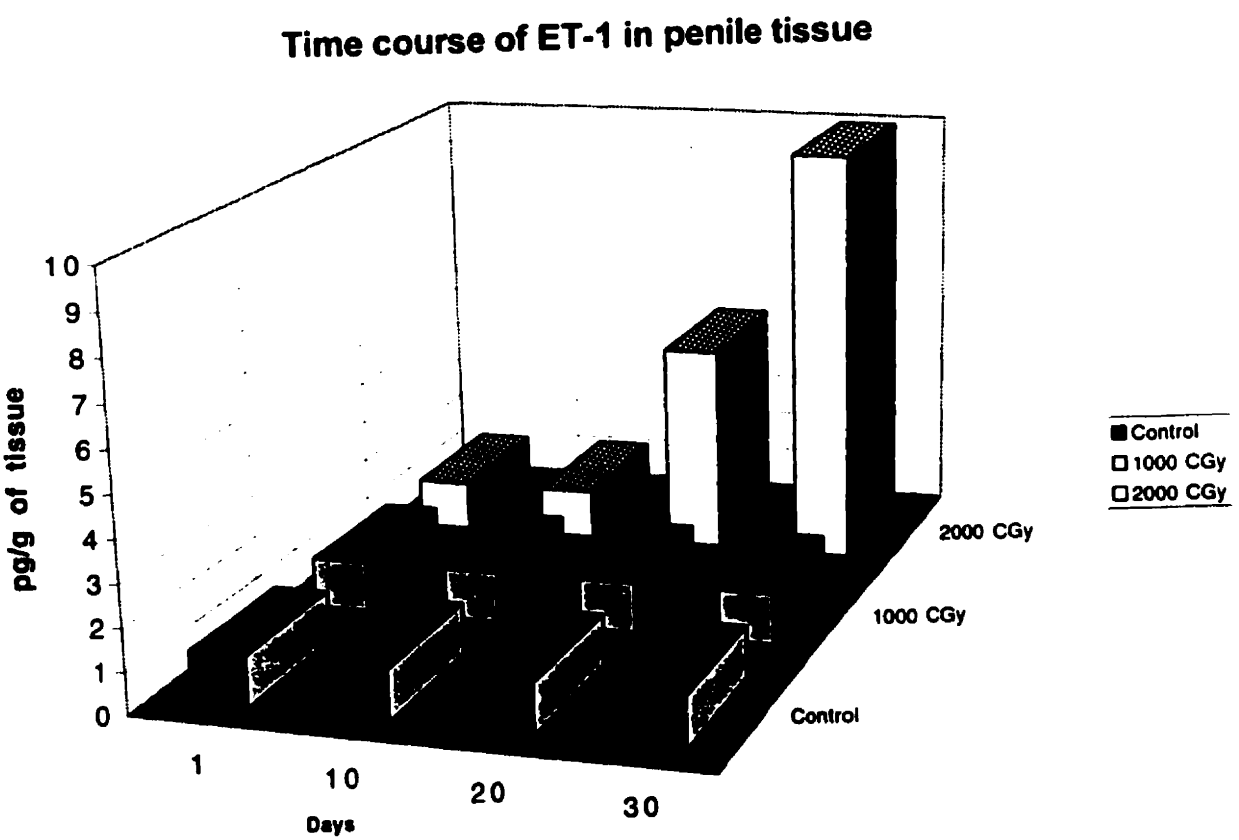

Figure 12 . Time course of ET-1 rise in penile tissue.

was a significant elevation of ET-1. At 20 days, the measured levels of ET-1 was 4.8 pg/g tissue±1.8, and at 1 month, it rose even further up to 10 pg/g tissue±4.3.

In prostatic tissue, the same pattern was seen in the control a nd 1000 cGy group. Both were equal in their levels of ET-1 at 3 pg/g tissue±0.8. In the 2000 cGy group, a similar pattern to penile tissue was seen. There was an increase in ET-1 levels only at 20 days and 1 month. At 20 days, the ET-1 levels were 8 pg/g tissue±2.1, whereas in at 1 month, it was at 15 pg/g tissue±3.1 (see Figure 14).

3.6 In vivo physiological assessment

Electrostimulation of the cavernous nerve revealed significant differences in the mean maximal intracavernosal pressure among groups. Before injection of the ET-1 antagonist (BQ-123), the control group has a significantly higher mean maximal intracavernosal pressure than that of the two irradiated groups. Before injection, the mean maximal intracavernosal pressure was 64.4 cm H2O compared to the 1000 cGy group (46.8 cm H2O) and the 2000 cGy group (45.2 cm H2O). There was no significant diference between the two irradiated gruops before injection of the antagonist. After injection, the control group was, at all time points, significantly higher than that of the 1000 cGy group. However, after injection, the 2000 cGy was significantly higher than the 1000 cGy, and control groups up until 90 minutes after injection. After the two hour electrical field stimulation, the mean maximal intracavernosal pressure of the 2000 cGY was no longer significantly different.

Figure 13. Systemic and intracavernosal pressure in an irradiated rat from the 2000 cGy group before injection of the ET_A antagonist (BQ-123).

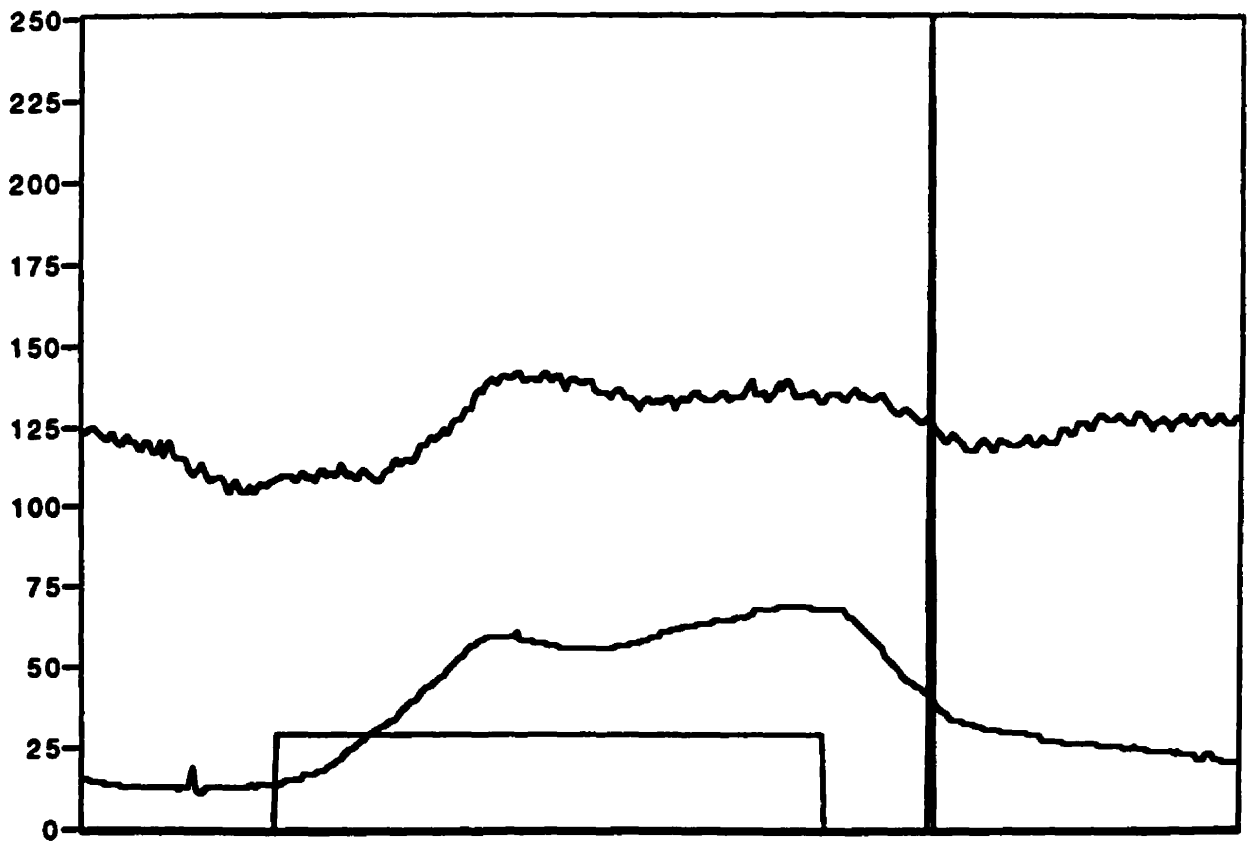

Figure 14 . Systemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 10 minutes after injection of the ET_A antagonist (BQ-123).

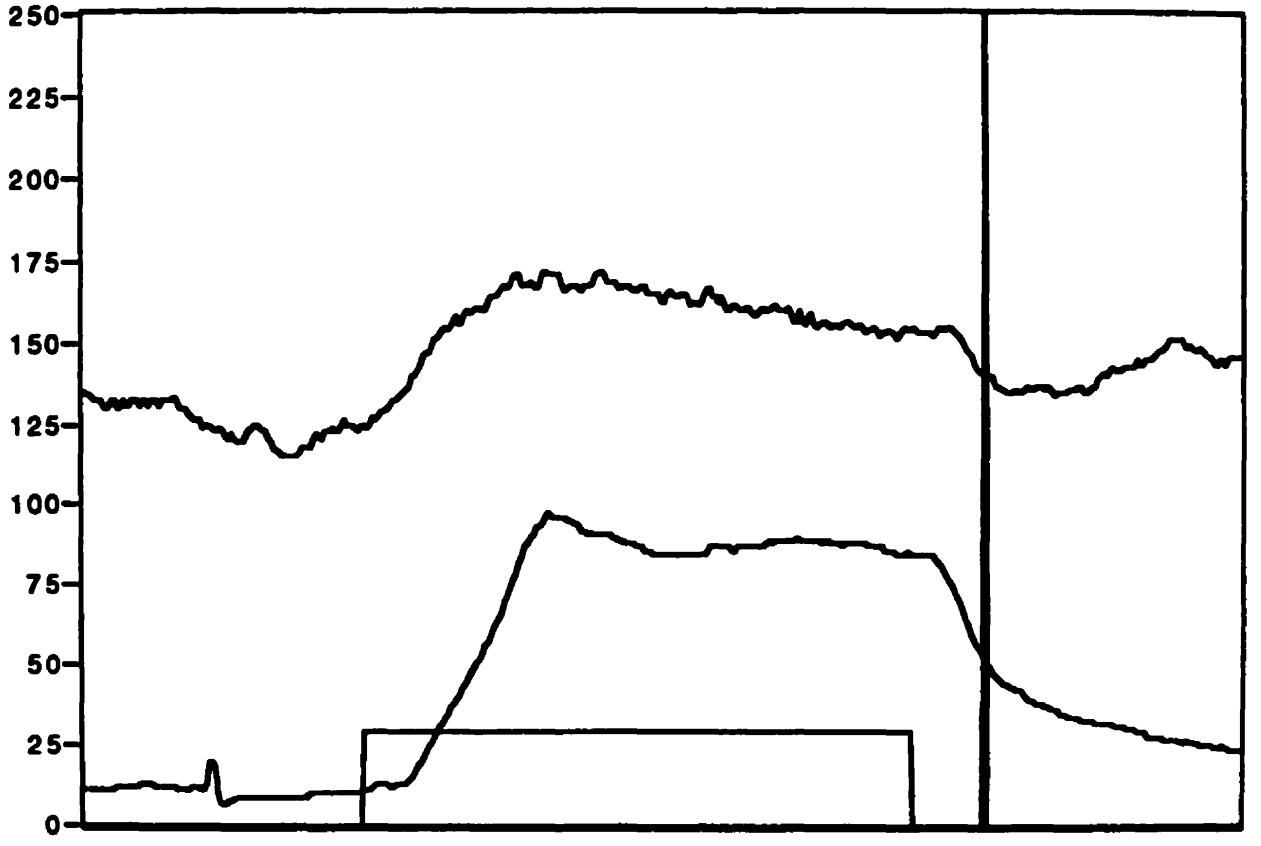

Figure 15 . Systemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 20 minutes after injection of the ET_a anyagonist (BQ-123).

Figure 16 . Systemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 30 minutes after injection of the ET_a antagonist (BQ-123).

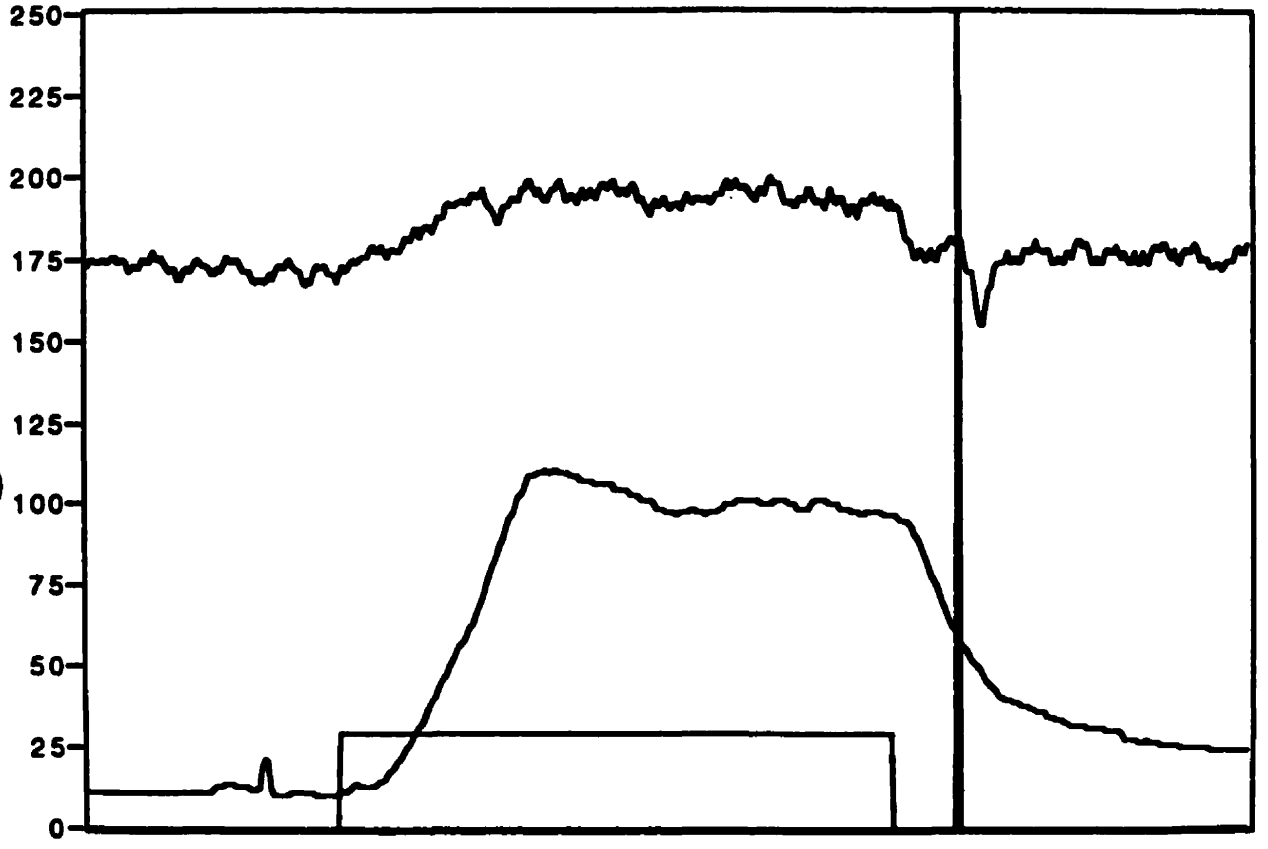

Figure 17 . Sytemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 40 minutes after injection of the ET_a antagonist (BQ-123).

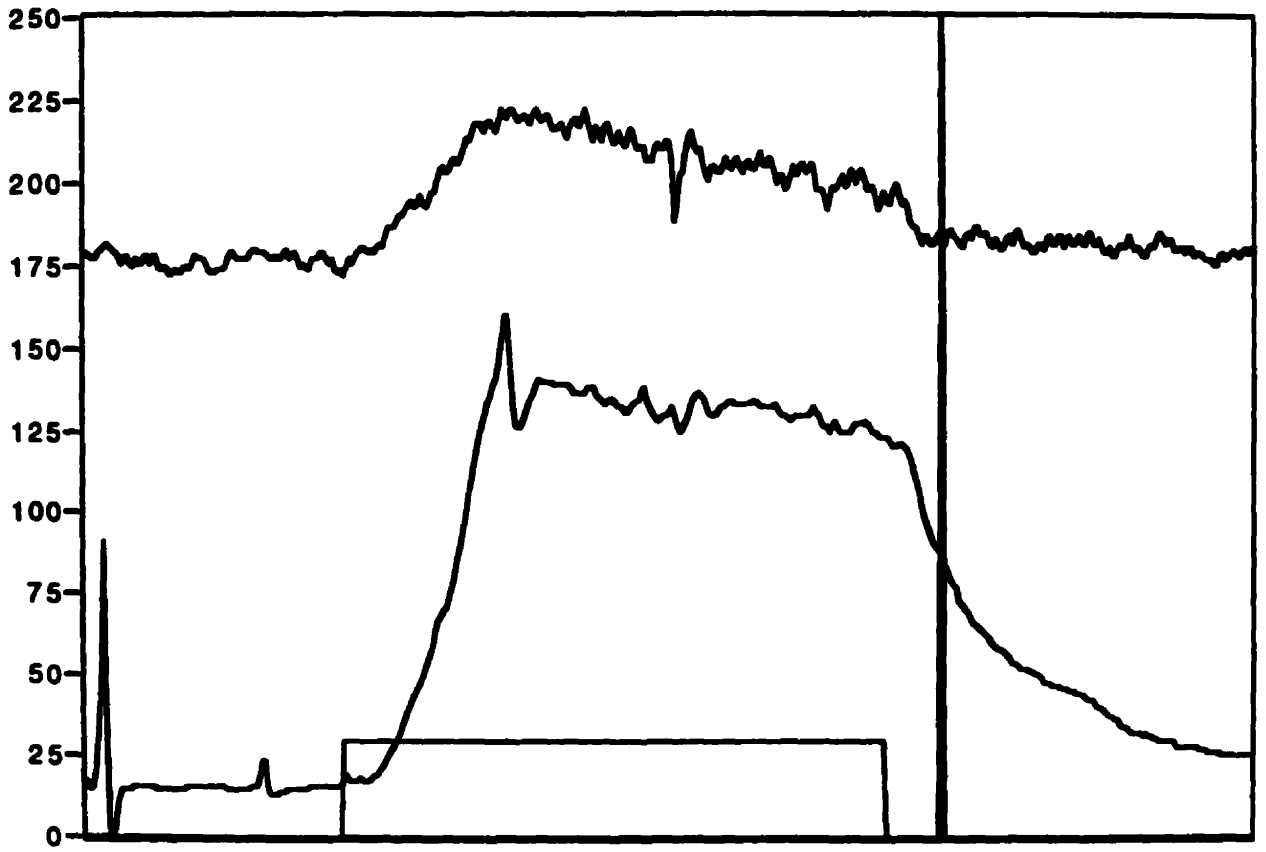

Figure 18 . Systemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 50 minutes after injection of the ET_A antagonist (BQ-123).

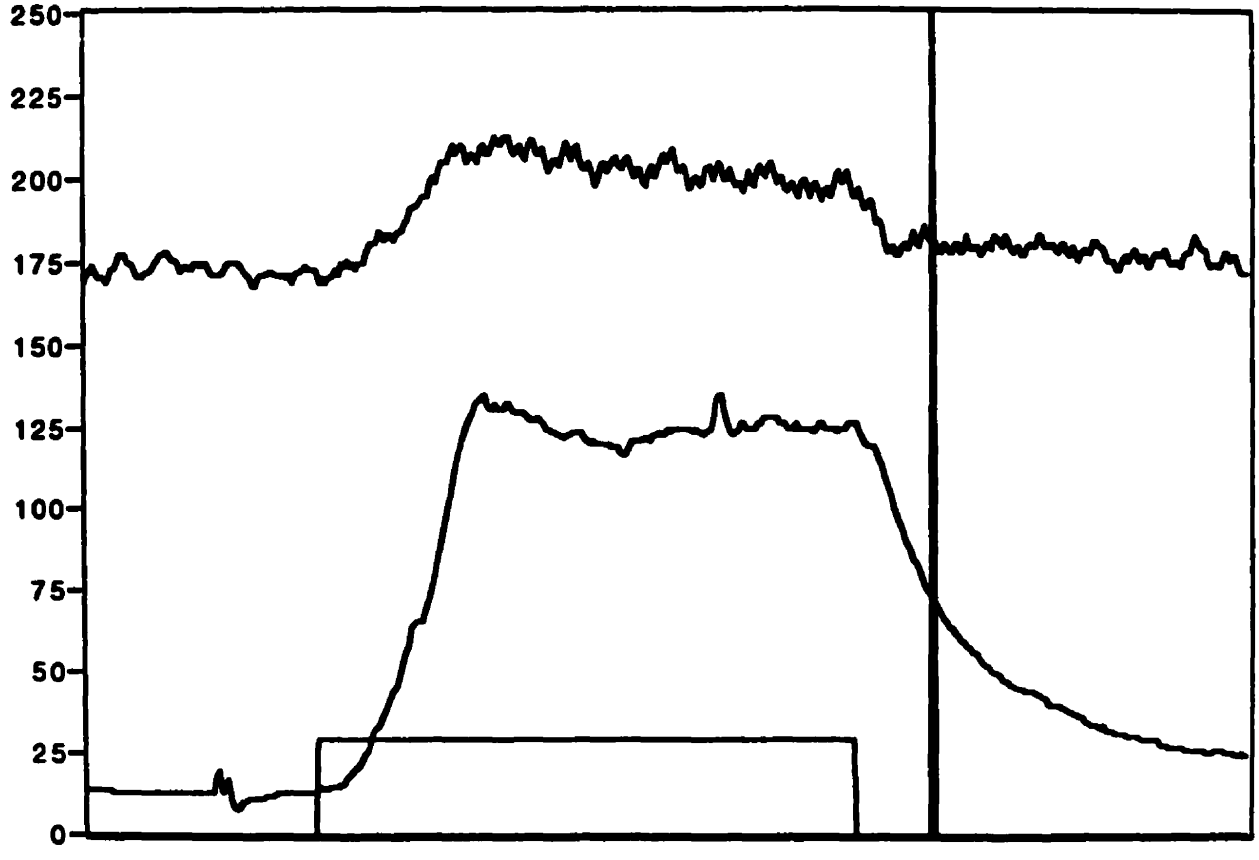

Figure 19 . Systemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 60 minutes after injection of the ET_a antagonist (BQ-123).

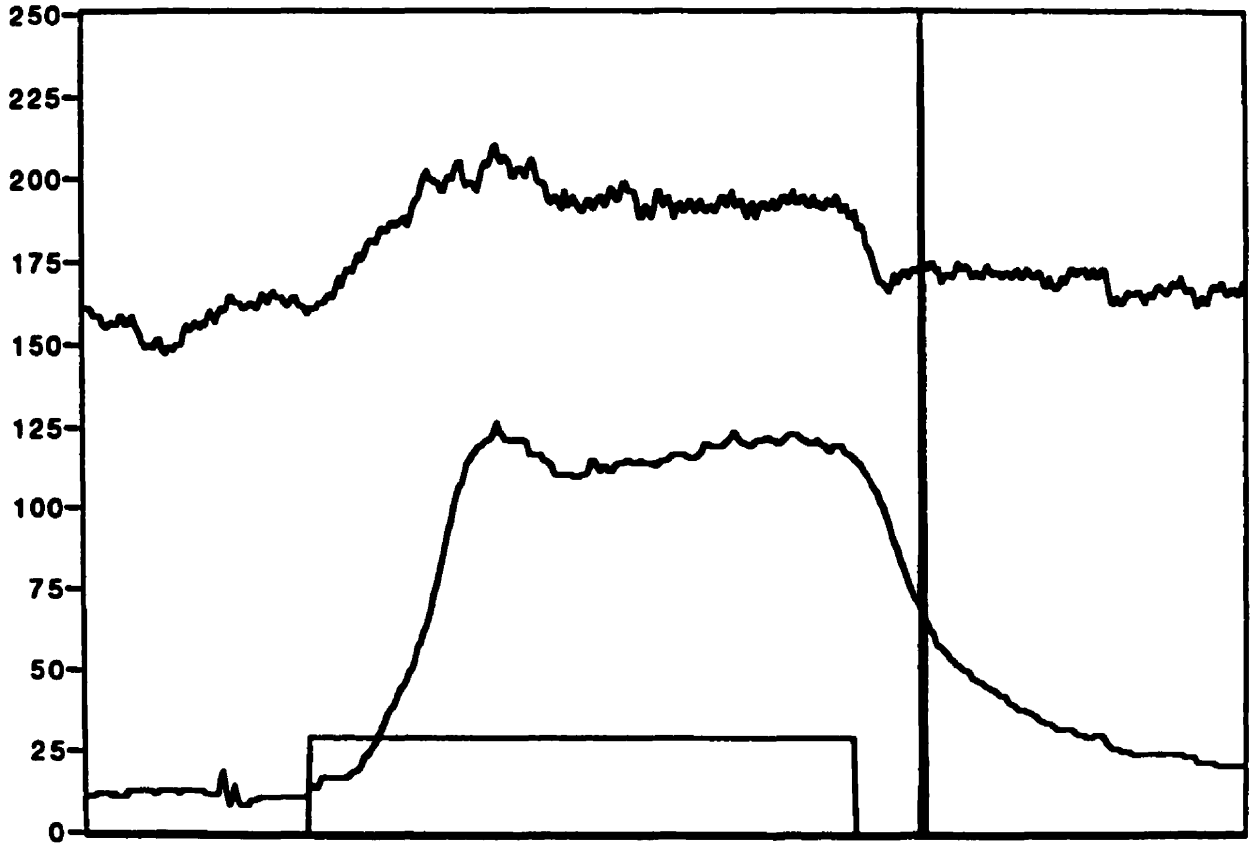

Figure 20 . Systemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 70 minutes after injection of the ET_a antagonist (BQ-123).

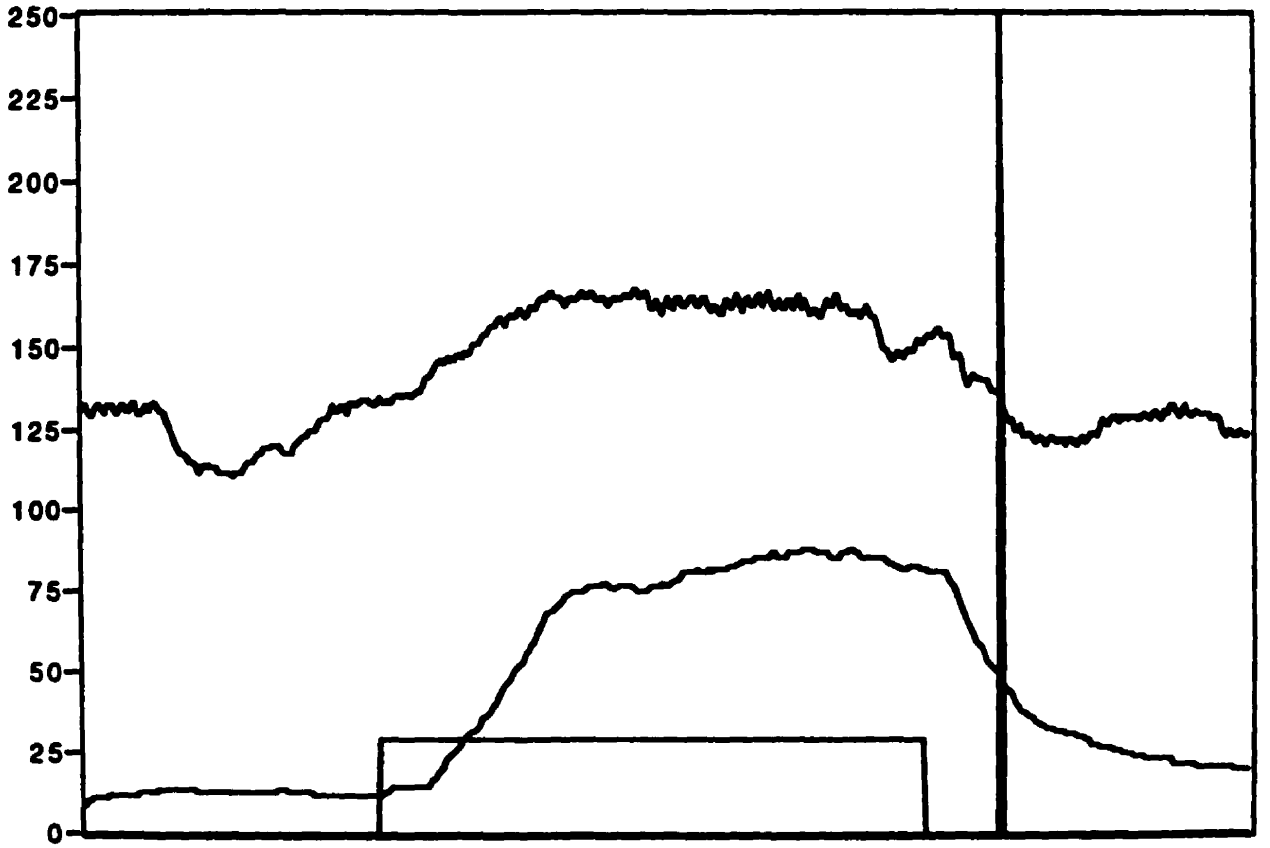

Figure 21 . Systemic and intracavernosal pressure recording in an irradiated animal from the 2000 cGy group 80 minutes after injection of the ET_A antagonist (BQ-123).

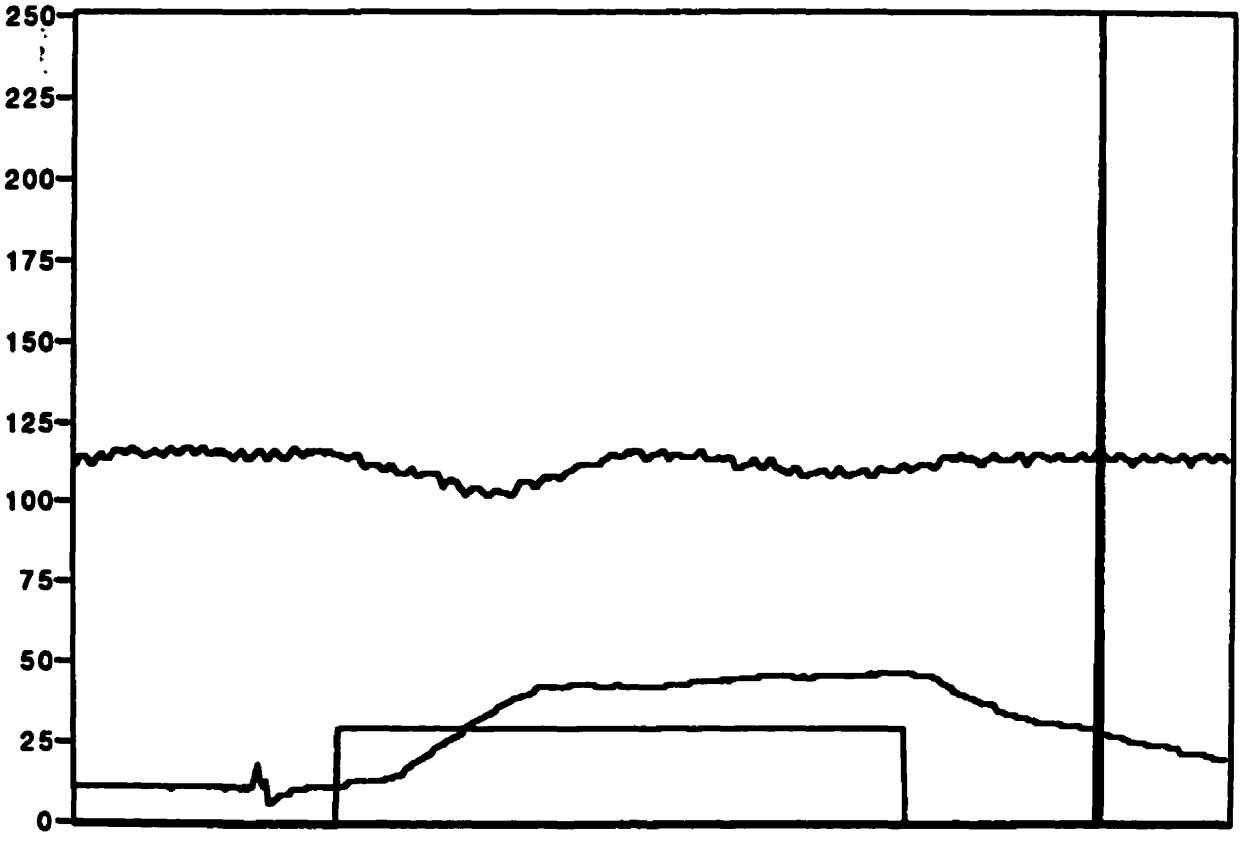

Figure 22 . Systemic and intracavernosal pressure recording 90 minutes after injection of the ET_A antagonist (BQ-123).

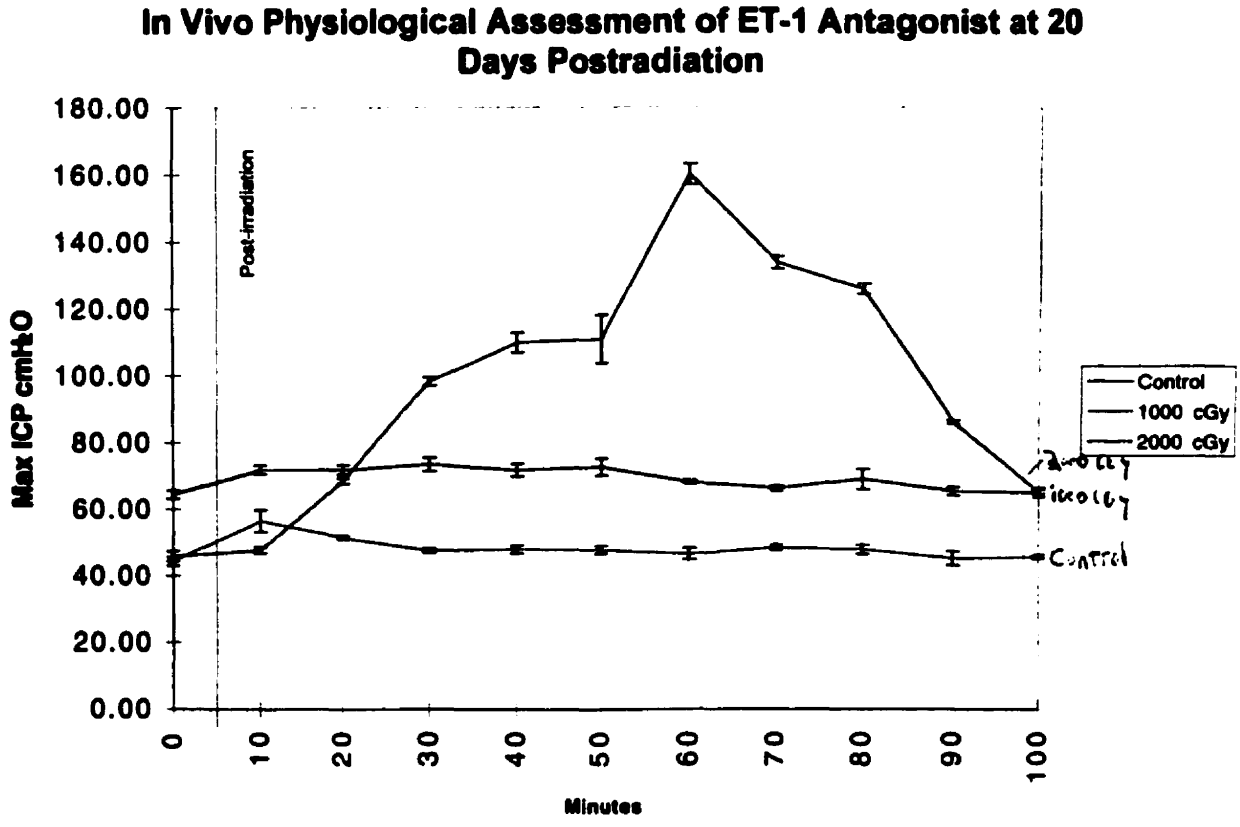

Figure 24 . Maximal intracavernosal pressures in the control, 1000 cGy, and 2000 cGy groups 20 days postradiation.

DISCUSSION

It is estimated that 30 million North American men have consulted with their physicians requesting medical therapy for erectile dysfunction[1]. Although regarded as a benign disorder, impotence often has a profound impact on the quality of life of many men. Over the past decade, dramatic advances in our ability to diagnose and treat this condition have been achieved through an improved understanding of the physiology of erection.

Studies evaluating the pathogenesis and etiology of impotence has been an active area of research over the past decade. Based on this work, our understanding of the basic physiologic mechanisms responsible for normal erectile function has been refined and more completely elucidated[35,36,37]. These advances in our knowledge have lead to improved surgical techniques, less invasive treatment options for men with erectile dysfunction and a greater understanding of how disease states alter the normal physiologic pathways of erection. Studies using animal models as well as those in man have demonstrated the neurovascular basis for penile erection[40,41].

Over the past few decades, research in impotence has focused mainly on the tumescence phase of erection, with nitric oxide being the principal mediator of erection[166]. However, in the last few years, attention has begun to focus on the delicate balance of vasodilator and vasoconstrictor substances neccessary to maintain vascular tone[167]. ET-1, a potent vasoconstrictor, has recently gained widespread attention as a mediator of tone in the systemic, pulmonary and penile systems[40,41].

4.1 Endothelium as an endocrine organ

Recent findings that endothelial cells are capable of producing numerous vasoactive substances has propelled this thin unicellular endothelial layer into a unique position as an endocrine, paracrine, and even autocrine organ[163]. In the penis, this thin

layer is in part responsible for the maintaining delicate balance between vasoconstriction and vasodilatatory substances. Furthermore, this thin layer is responsible for the crosstalk network between endothelium-derived substances. Endothelial cells release both potent vasoconstrictor (ET family of peptides), and an extremely potent vasodilator (NO)[110]. Previous studies have shown the importance of endogenous synthesis of ET-1 contributing to the maintenance of a basal vascular tone in humans, acting at least in part through ET_a receptors. However, the physiological role of ET-1 in maintaining the tone of the penile cavernosal smooth muscle in the flaccid state and their interaction with NO/guanosine 3'-5' cGMP axis is not fully understood. However, it has been suggested that any alteration of this delicate equilibrium could lead to a substantial alteration in blood flow. Thus, the role of the endothelium as an endocrine organ is of paramount importance in our understanding the physiology and pathophysiology of erection. Studies in which this layer has been denuded provide strong evidence for an important physiologic role. Disease processes such as atherosclerosis, diabetes and others may be in part manifest by differences in the endothelial layer.

4.2 The role of ET-1 in advanced prostate cancer

Prostate cancer is currently the most commonly diagnosed cancer in Canadian men. It is estimated that 15 000 Canadian men will be diagnosed with prostate cancer in 1997, and 4200 will die from this disease[8]. The incidence of prostate cancer is rising partly as a result of increased screening and as a reflection of the increasing average age on the population.

ET-1 has been shown to be involved in the pathophysiology of prostate cancer tumor biology[165]. Recently, it has been shown that both plasma ET-1 and the ET_a receptor are elevated in advanced prostate cancer[165], the ET_b receptor is decreased. ET-1 increases alkaline phosphatase activity in new bone formation[165], indicating that ET-1 may be a mediator of the osteoblastic response of bone to metastatic prostate cancer[173].

Furthermore, plasma -immunoreactive ET-1 concentrations have been shown to be elevated abnormally in 58% of men with metastatic tumors[166]. Human prostate cancer cell lines have been shown to universally produce ET-1 at the level of mRNA and protein.

ET-1 is a growth regulatory peptide. ET-1 can influence cell proliferation directly and has potent synergy with many of the same peptide growth factors implicated in advanced prostate cancer progression. ET-1 may act as a facilitator of the more permissive growth factors such as platelet-derived growth factor (PDGF) and epidermal growth factor (EGF). This potentiation by ET-1 may be an important mechanism of androgen-independent prostatic cancer growth.

In addition to its role in advanced prostate cancer, ET-1 has now been shown to be altered in treatment modalities used for prostate cancer. In this study, we have examined if exposure to radiation produced an increased amounts of ET-1 like-immunoreactivity in both the penis, prostate, and in the systemic circulation. Furthermore, using an in vivo rat animal model, we evaluated whether an ET-1 antagonist reversed the loss of erection secondary to radiation in vivo, using a rat animal model. This study supports a previous study which showed a significant decrease in the NOS-containing nerve fibers within the penis following radiation treatment[23]. Thus, these studies demonstrate the importance of the delicate balance of vasoactive substances being released from the endothelium. It is possible that NO regulates ET-1 synthesis and/or secretion together with ET_A receptor expression. This proposed co-ordinated reciprocal regulation of these vasoactive compounds has been noted previously by Kuchan [165] using shear stress on cultured human umbilical endothelial cells. Any alteration of NO could upset the amount of ET-1 being secreted. Furthermore, these studies demonstrate the antagonistic vascular effects between these two substances. This study suggests that radiation treatment leads to an increased ET-1, decreased NOS level leading to erectile dysfunction. This pattern is seen in other

tissues such as the lung. In hypertension, immunohistochemistry showed in pulmonary tissue to have an increased ET-1, decreased NOS level.

4.3 Testosterone levels

In order to determine that erectile dysfunction was not due to a testosterone deficit, we measured the levels of tetosterone following radiation treatment. Our results showed that the decrease in intracavernosal pressure noted following radiation treatment was not due to an altered hormonal environment. Our results have shown that the testosterone levels are constant, independent of the radiation dose received. Our shielding of the testes during the treatment was effective. Furthermore, the testes were evaluated histologically to determine whether there were any pathological changes as a result of the radiation. Our data did not show any pathological alteration. As a result of the above findings, we believe that the erectile dysfunction seen in the rat animal model is likely a result of the increased ET-1 levels.

4.4 Plasma levels of ET-1

Initially in this study, our primary objective was to assess whether ET-1 played a role in RAI. We quantified ET-1 on a time course following radiation treatment to establish whether ET-1 increased after RAI, in both prostatic and penile tissue and in the systemic circulation. In the blood, our results showed a significant increase 10 days following radiation at 2000 cGy. No significant difference was observed at 1000 cGy. This could be due to the fact that not enough radiation was given to cause any pathological alteration. As well, perhaps at this low dosage, there is reperative effects, so that there a slower time course. This increase of ET-1 at 2000 cGy could cause an increased vasoconstriction and could contribute to impaired erectile function seen in our rat animal model. This increased ET-1 post-irradiation levels suggest a possible role in RAI in which increased corporal smooth muscle contractility or an impaired corporal smooth muscle relaxation is present. Because it was shown in a previous study[23] that RAI decreases the level of NOS in the nerves that innervate the penis, and because NO

could regulate the expression of ET-1, perhaps RAI might involve a loss of negative feedback. Furthermore, this increased serum ET-1 could be an important factor in the accelerated atherosclerosis seen in patients.

4.5 Tissue levels of ET-1

Our results showed an increase in ET-1 levels in the prostate and penis 20 days, 1 month, and 2 months following 2000 cGy. No significant increase was seen at 1000 cGy. These increased ET-1 levels could potentiate atherosclerotic lesions by acting as an autocrine growth factor for smooth muscle cells in the corpus cavernosum. ET-1 has been identified in sections of atherosclerotic human arteries. Positive immunostaning for ET-1 has been localized in atherosclerotic plaques and in areas of neovascularization[76]. This has important implications since atherosclerosis is a major contributing cause for vasculogenic impotence. One reason for such changes is the fact that the hypogastric-cavernosal arterial bed is unique. The resting flow in the cavernosal artery is approximately 10 mL/min and increases sixfold during sexual intercourse[14]. Few other vascular beds are required to function over such a wide range of flows, especially in a 50 -year-old man. Thus, this delicate balance of vasoactive substances must be kept in order for normal physiological function to occur. Previous studies have supported a role for ET-1 as a autocrine frowth factor via its receptors, especially the ET_b receptor[69]. Furthermore, ET-1 stimulated DNA synthesis, only in the presence of the Endothelin-Converting Enzyme (ECE)[70].

4.6 Interpretation of the in vivo asessment

A physiological study was as well carried out to validate our hypothesis that radiation alters erectile function, and that the use of an ET_a receptor antagonist could potentiate electrical field stimulation cavernosal penile rise in a rat animal model. It is believed that part of the underlying pathology responsible for RAI is of vascular origin. We believe that upon radiation exposure, there is an increased release of ET-1, which reduces the blood supply to the penis. Increased amounts of ET-1 could aid in

producing erectile dysfunction by either altering the capacity of the smooth muscle to relax or by inducing pathological changes in the pelvic vasculature. It is also known that other vascular risk factors such as cigarette smoking, hypertension, atherosclerotic diet, diabetes, and family history are important contributing causes that could increase the incidence of post-radiation impotence[14].

Our in vivo rat animal model showed that intercavernosal pressures in irradiated rats were significantly decreased compared to controls. When these rats were given BQ-123, an ET_a receptor antagonist, the erectile function of these rats were significantly potentiated. Because we were able to potentiate erectile function in these rats, these results offer some insight into the pathophysiology of RAI. Whether this increased ET-1 acts on altered ET receptors has yet to be determined. However, this increased concentration of ET-1 levels provides insight into the arteriogenic impotence seen in RAI. We could also make some parrallel comparison to understand the arterial problems occurring in women irradiated for breast cancer. These problems could be overcome by substances blocking the effects of ET-1. Recent reports demonstrating persisting improved arterial flow among men using intracavernous agents for arteriogenic component of impotence has provided evidence to justify the early use of vasoactive therapy[165]. This form of therapy may serve to up-regulate the nitric oxide synthase within the cavernous nerves and endothelium.

Perhaps in the future, one of the vasoactive agents that will be used to inhibit the actions of ET-1. What would be useful is an inhibitor which can resist proteolytic degradation, such as a non-peptide antagonist.

4.8 Future direction

This study is essentially concerned with mechanisms underlying penile dysfunction after irradiation and the potential role played by ET-1. This study highlighted the physiologic role of ET-1 and the significant modulation in both

circulating and tissue levels, seen following radiation. Use of a specific blocker of the Et_a. receptor, BQ-123, able to antagonize the vasoconstricting effects of ET-1 supported the concept of an important role for these vasoactive agents in erection However, further studies must address the role played by the ET receptors in RAI. The role played by the ET_b is important as this receptor has a dual role. On the endothelial cells, it mediates vasodilation, while those on the smooth muscle cells mediate vasoconstriction. Insight into the role played by this receptor could lead to many important answers in the pathophysiology of this common complication. If the ET_b relaxation is deficient, the vasoconstrictor role will prevail. This could lead to increased contractile effects, as seen in our study. Furthermore, future studies should focus on the role played by the ECE. Using inhibitors to this enzyme, further insight into the role of ET-1 can be elucidated. Perhaps future clinical work on vascular insuficiency could include (ECE) inhibitors, in the same regard as angiotensin-converting enzyme (ACE) inhibitors are important in hypertensive therapy. Use of a rabbit model able to mimic the atherosclerosis seen in patients would serve as an extremely useful model to assess the role of ET-1 as a growth regulatory peptide in RAI.

Future work using fractionated dosages of radiation over a longer time period may more closely reproduce the human experience. Thus, differences could be established between the acute phase and chronic phases of this complication.

This study can be done clinically, as well. Our study can assess patients undergoing therapy for prostate cancer. Doppler analysis of penile blood flow and blood analysis for ET-1 can be performed before and following radiotherapy, at different time points. This clinical study will allow us to determine if the results seen in the rat model is applicable to man.

Based on the results of this study , new clinical questions emerge. For example, should a patient with prostate cancer and a history of vascular risk factors reconsider other options such as radical surgery? Should microsurgical penile

revascularization be considered as a therapeutic option? Prospective clinical studies will be needed to determine the validity of these proposals.

4.9 Conclusion

In conclusion, normal erectile function is characterized by a delicate balance between the effects of vasoconstricting and vasorelaxing agents. To achieve an erection adequate for sexual intercourse, an intact neurovascular mechanism must be present. Pelvic radiotherapy damages both vascular and neural components.

The bulk of current medical literature supports the concept that radiotherapy produces a primary effect on the vasculature which is progressive over the course of months to years in man.

The future holds promise of new antagonists, able to modify the vasoconstricting actions of circulating agents, coupled with an improved understanding of the anatomy and physiology of erection. Great strides have been realized over the past 15 years. With these new developments, even more exciting times lay ahead.

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