THE IMPORTANCE OF NEURAL FACTORS IN THE PRESENTATION AND TREATMENT OF PROSTATIC OBSTRUCTION.

CHRISTOPHER R. CHAPPLE BSc., MBBS, FRCS.

Submitted to The University of London for the degree of Doctor of Medicine.

ABSTRACT

Symptomatic bladder outlet obstruction due to prostatic enlargement is a common problem in urological practice. Hyperactive detrusor function, "detrusor instability", occurs in up to 80% of patients presenting with prostatic obstruction and in most cases it resolves post-operatively when the obstruction has been relieved. This dysfunction is generally regarded as a modern concept but, in 1786, John Hunter recognised the complex nature of prostatic obstruction and reported: "The disease of the bladder arising from obstruction alone, is increased irritability, and its consequences, by which the bladder ...becomes quick in its action and thick and strong in its coats."

Animal models have confirmed the relationship between obstruction and instability. Several hypotheses have been proposed to explain this link and include; (1) post-junctional hypersensitivity - possibly related to denervation, (2) altered adrenoceptor function, (3) afferent nerve dysfunction, (4) an imbalance of peptide neuro-transmitters, and (5) a primary or acquired myogenic deficit.

The principal motor control of the intraprostatic musculature is mediated by the sympathetic nervous system; however, the mechanism of action and specific localisation of prostatic adrenoceptors and the importance of non-adrenergic neurotransmission in man is poorly understood.

A study of patients with symptomatic prostatic obstruction was undertaken to investigate the influence of neural pathways in determining the pathogenesis and clinical presentation of prostatic and detrusor dysfunction. Sixty-two patients were investigated using modern urodynamic techniques and sub-divided into three groups; control, stable obstructed and unstable obstructed. Biopsies of prostate, bladder neck and bladder muscle were taken at the time of surgery, and pharmacological, autoradiographic and histochemical studies performed. The prostate, bladder and bladder neck were found to be innervated by a complex network of noradrenaline-, acetylcholine-, neuropeptide-, and amine-containing nerves. Separate quantitative analyses of these neurons were carried out and corrections applied to compensate for muscle hypertrophy and hyperplasia. The histological findings were complemented wherever possible by biochemical assay of neurotransmitters.

There was a significant reduction in the acetylcholinesterase positive innervation of the obstructed bladder compared with control, which was most marked in tissue from patients with detrusor instability. A similar reduction in the non-adrenergic, non-cholinergic sensorimotor neurotransmitters was evident. Biochemical changes within the detrusor included an increase in noradrenaline content and a decrease in the putative sensory neurotransmitter substance P.

Detrusor muscle strips from obstructed patients showed increased contraction in response to acetylcholine, suggesting that denervation hypersensitivity might contribute to the pathogenesis of post-obstructive detrusor instability. Normal detrusor muscle relaxed in response to

noradrenaline. In contrast, detrusor muscle from unstable obstructed patients contracted; a response most marked in detrusor muscle from patients who had presented in acute retention.

In vitro prostatic muscle-strip experiments confirmed that contraction of prostatic muscle is produced by α_1 adrenoceptor stimulation. Radioligand binding assays endorsed the results of these experiments by demonstrating a clear excess of α_1 receptors over α_2 receptors in histologically normal and adenomatous prostate. Auto-radiography showed the precise localization of the two types of adrenoceptor and confirmed the predominance of α_1 receptors within prostatic musculature.

The complexity and potential importance of the autonomic nervous system in the pathogenesis and symptomatic expression of prostate-mediated bladder outflow obstruction, is demonstrated by this work. Marked changes in the innervation of both the prostate and bladder accompany obstructive benign enlargement of the prostate. The characterisation and localisation of the prostatic adrenoceptor is of considerable relevance since it validates the therapeutic use of selective prostatic α_1 blockade in the clinical management of obstructed patients. Preliminary immunohistochemical studies of bladder, bladder neck and prostate are presented, which provide an histological basis for further functional investigative studies.

As men draw near the common goal,

Can anything be sadder,

Than he who, master of his soul,

Is servant to his bladder.

ANONYMOUS.

This work could neither have been carried out nor completed without the selfless encouragement and understanding of my wife Mary, to whom it is dedicated.

ACKNOWLEDGEMENTS

The work reported in this thesis would not have been possible without the support and advice of my consultants Mr Euan Milroy and Mr Richard Turner-Warwick, who allowed me to study patients under their care. In particular, I am indebted to my university supervisor Professor M. Hobsley for his guidance and help with the preparation of this thesis and to Professor G. Burnstock and Professor J. Gosling for scientific advice and the use of facilities within their departments. This work was inspired by previous animal studies looking at the patho-physiological effects of urethral obstruction on the animal bladder.

All of the experimental work carried out in this study was instituted and supervised by the author, but would not have been possible without the guidance and collaboration of a number of scientists. I wish in particular to acknowledge the following:- Dr Helen Moss for instructing me on the principles of in vitro isometric muscle strip studies and carrying out subjective quantification of bladder and bladder neck neuropeptides; Dr Sally Ann Gilpin for her guidance on the principles of objective nerve counting and for carrying out assessments of muscle cell size; Dr Michael Davey for his support and inspiration with the prostatic pharmacological studies and instruction on the subject of α-adrenoceptors and Mr Michael Aubrey in collaboration with whom I carried them out; Dr Sharon James whose technical expertise and hard work made the autoradiographic studies possible; Dr Pam Greengrass for carrying out test-tube ligand binding studies; Dr Rahima Crowe for carrying out the subjective quantification of prostatic peptide immunoreactivity; Dr Pam Milner and Dr Jill Lincoln for carrying out the biochemical assays of peptide neurotransmitters and noradrenaline tissue levels. Much of this work would not have been possible without the technical help of Marie Phillips and the laboratory staff of Professor Gosling's department.

I am indebted to Miss Caroline Doyle for her secretarial assistance and Mrs Denise Abbott for editorial assistance. The photographic work was largely carried out in the Department of Medical Photography at The Middlesex Hospital with the help of Mr Eric Cheung and Mr Ray Phillips and their staff.

These studies were started whilst I was research fellow and subsequently completed during my tenure of senior registrarship in the Department of Urology at The Middlesex Hospital.

INDEX.

CHAPTER 1

INTRODUCTION

The Clinical Problem and an outline of this thesis

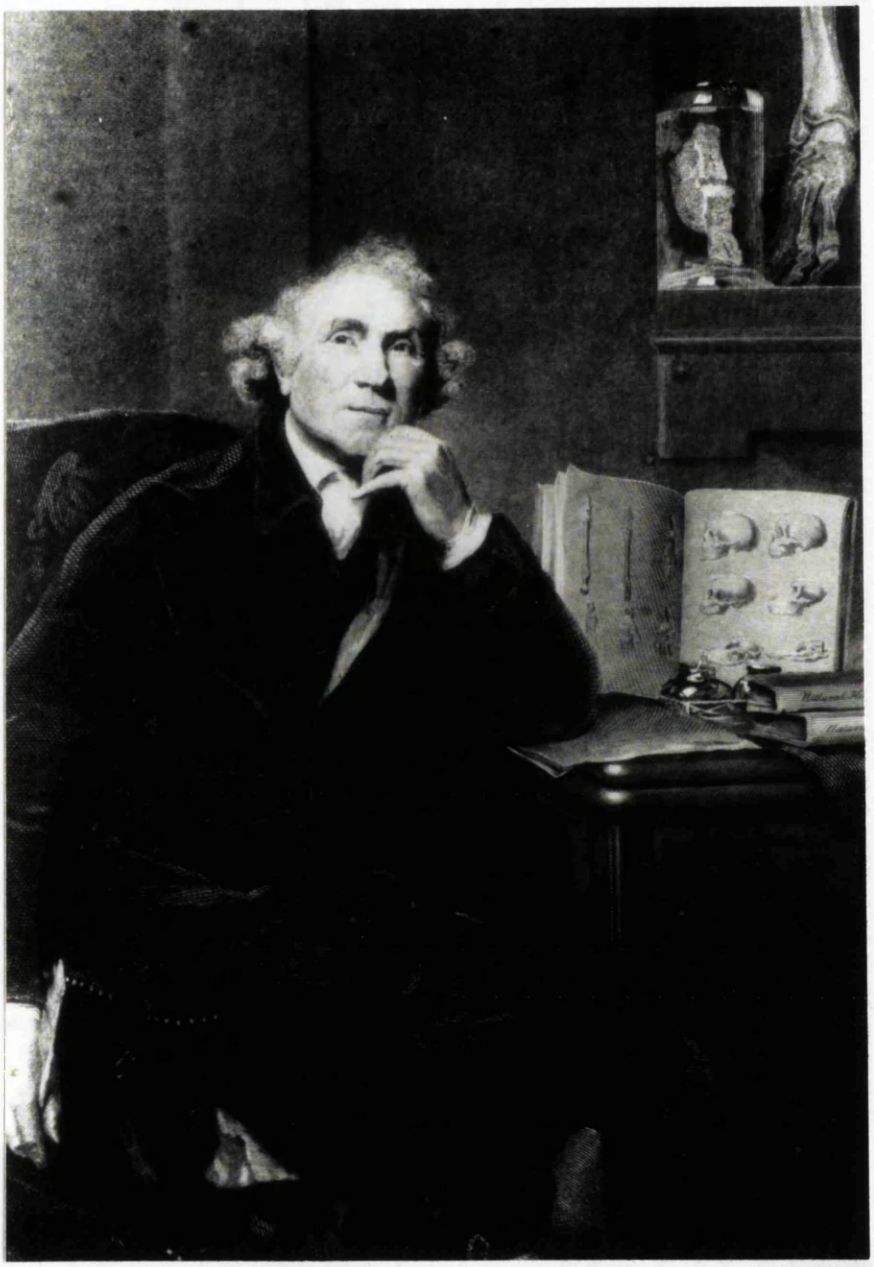
1.1 Historical Review

Male bladder outflow obstruction has presented a clinical problem throughout medical history. Catheters were used by the ancient Egyptians and Chinese as treatment for acute retention (Murphy, 1972). Jean Riolan the younger (1577-1657) is credited with being amongst the first to suggest that prostatic enlargement could result in mechanical obstruction to the bladder outflow tract (Shelley 1965).

Morgagni (1769) reported that "the swelling of the prostate is most common in the decline of life". John Hunter (Figure 1.1) in his "treatise on venereal disease" (1786) first recognised the structural and functional implications of lower urinary tract obstruction. He described the necessity for hormonal factors derived from the testis to the normal structure and function of the prostate and noted the asymmetric nature of prostatic enlargement. He presented such a clear synopsis of the functional problem that it is worth quoting it in its entirety "... the lateral width of the urethra gives such a resistance to the force or power of the bladder in expelling the urine as is easily overcome by the natural action of the bladder; but when the canal is lessened, either by stricture, spasm, swelled prostate gland or any other means, this proportion is lost, by which means the bladder finds greater difficulty than normal and is of course thrown into an increased

Figure 1.1.

John Hunter in his library (courtesy of the Wellcome Library).



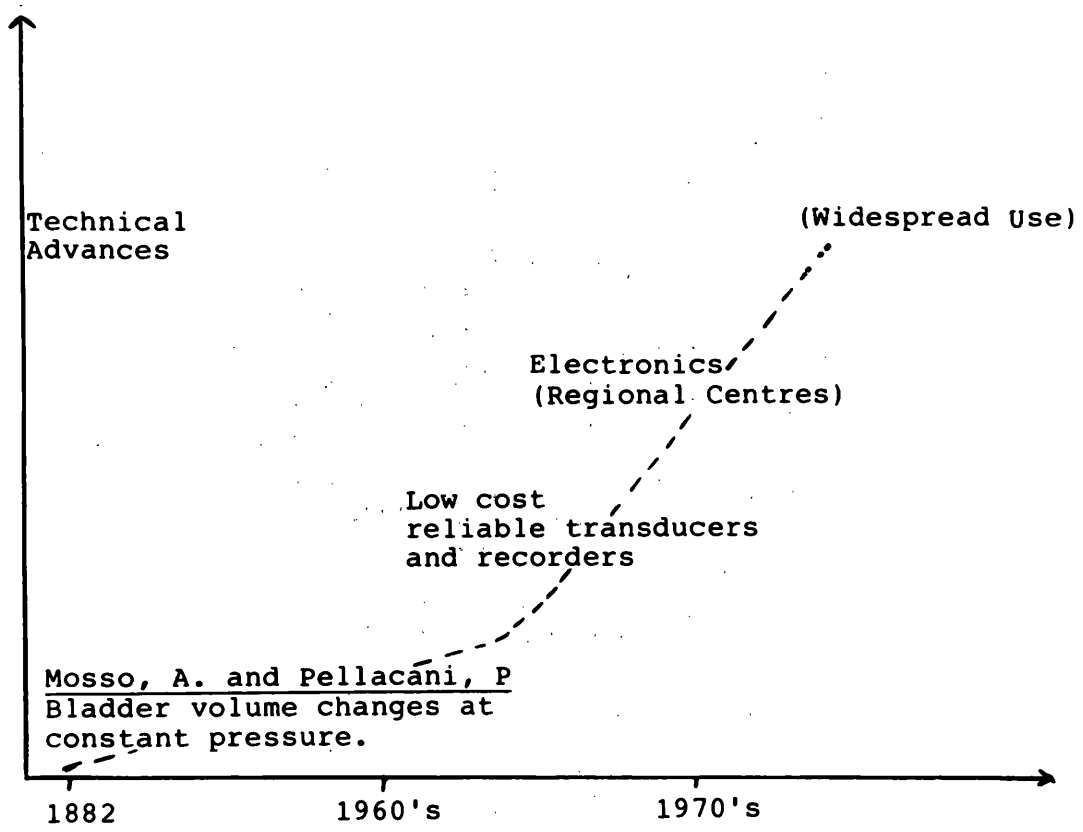
action to overcome the resistance, which becomes a cause of the irritability and increased strength of this viscus in such diseases ... the disease of the bladder arising from obstruction alone is increased irritability and its consequences, by which the bladder admits of little distension, becomes quick in its action and thick and strong in its coats". Despite providing this marked insight into the clinical problem such ideas were not followed up by other workers (c.f. Thompson 1860) for nearly two centuries.

1.2 Urodynamic investigation.

The introduction of technology enabling measurement of intravesical pressure during filling (Dubois,1876) and voiding (Mosso and Pellacani 1882) laid the foundations for the development of techniques to study both normal and disordered bladder function (Figure 1.2). Rose (1932) reported an increased intravesical pressure during filling cystometry in patients with prostatic obstruction. However, the ability to measure pressure and flow parameters awaited the development of strain gauge transducers (Von Garrelts, 1956, Smith 1968, Claridge 1966). Smith (1968) clearly defined the pressure/flow relationship in obstruction and suggested a mathematical relationship (maximum voiding detrusor pressure/ maximum flow rate) for the calculation of urethral resistance. Claridge (1966) suggested that many of the symptoms of obstruction were due to changes in the detrusor; he noted that the intravesical pressure at rest prior to micturition was higher in obstructed patients and concluded that frequency and urgency were related to this increased pressure. Hodgkinson (1963) had previously

FIGURE 1.2

Diagrammatic representation of the time scale underlying the development and distribution of urodynamic technology.



reported similar bladder over-activity in a group of women presenting with urge incontinence and noted the ability of a normal subject to inhibit such bladder contractions. In both groups of patients there were no demonstrable associated urological abnormalities, an important consideration since other workers had suggested that detrusor overactivity could be attributed solely to an underlying urological abnormality (Miller et al. 1965).

Bates (1970, 1971a, 1971b) defined the role of clinical urodynamics and from the unit at The Middlesex Hospital described the technique of synchronous cine/flow/cystourethrography (videocystometrography). He stressed the importance of measuring the true detrusor pressure (total bladder pressure minus abdominal pressure) and of carrying out such studies in both standing and lying positions in order to unmask any underlying abnormality (vide infra). In addition he clearly showed that if the bladder was filled at a rate of 100 mls/minute in "normal subjects" who were specifically asked to hold their urine, then there was little rise in detrusor pressure during filling, even when the patient felt uncomfortably full; the so called "stable" detrusor.

This group of workers applied the term "unstable" bladder (detrusor instability) to the over-active detrusor behaviour previously recognised by Hodgkinson in 1963. In the absence of a demonstrable neurological abnormality they noted its occurrence in a number of groups of patients; including up to 10% of the general population and as a secondary phenomenon in over 50% of those with proximal urethral outflow obstruction (Turner-Warwick

and Whiteside 1979). Based on this work urodynamics has now received widespread international acceptance. The techniques and terminology used in urodynamic investigation are monitored and standardised by the International Continence Society (Abrams et al 1988). Initial experience suggested that an involuntary detrusor pressure rise in excess of 15 cm H_2O was pathognomonic of detrusor instability. Subsequent work has suggested that an absolute value of this nature should be avoided and that the pattern of the pressure rise is more important (see Figure 1.3).

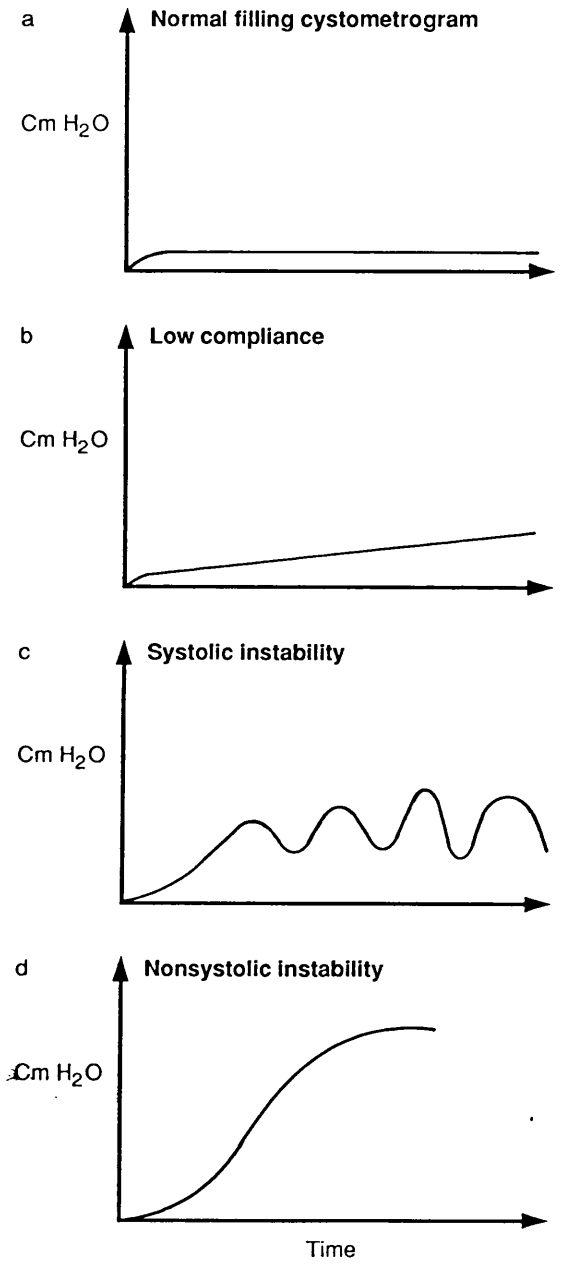
Controversy remains concerning the differentiation of marginal or "steep" instability from "low compliance" (Abrams et al. 1988); and indeed whether they might not represent the same phenomenon (see Figure 1.3). Compliance is defined as the change in volume for a given change in pressure. During normal bladder filling there is little or no pressure change, although the mural tension increases as the bladder fills (Smith 1976).

This phenomenon can be attributed to the physiological property of smooth muscle known as receptive relaxation; this is not nerve-mediated, but rather reflects the physiological and physical properties of the bladder wall (Tang and Ruch 1955).

Unfortunately, a barrier to resolution of the debate over the distinction between low compliance and detrusor instability is raised both by our lack of understanding of the pathogenesis of detrusor instability and our limited knowledge of the mechanisms underlying the normal function and, in particular, neural control of human detrusor smooth muscle. Therefore the

FIGURE 1.3

Stylised filling cystometry traces to demonstrate a) normal, b) low compliance, c) systolic instability, d) non-systolic instability.



majority of cases of detrusor instability remain idiopathic.

Further progress in our understanding of detrusor instability has awaited the development of a suitable model; in which an identifiable factor can be related to the subsequent onset of secondary detrusor instability. Investigation of prostatic bladder outflow obstruction provides this opportunity.

1.3 Prostate Obstruction and Detrusor Instability

A review of the literature (see Table 1.1) reveals that detrusor instability occurs in between 52 and 80% of men with bladder outlet obstruction due to benign prostatic hyperplasia. Conversely, surgical relief of bladder outflow obstruction results in a recovery of normal detrusor behaviour in the majority of patients (Table 1.2). Such observations lend support to the hypothesis that there is a causal link between the two conditions.

The scientific investigation of prostate obstruction using urodynamic principles has allowed the recognition of two main groups of symptoms :

1. Obstructive - hesitancy, poor stream, feeling of incomplete bladder emptying.
2. Irritative - daytime frequency, nocturia, urgency, and urge incontinence.

The normal male voids to completion at a maximum pressure of 40-50 cmH_2O and a free flow rate in excess of 25 ml/sec. In the initial stages of bladder outflow obstruction an increase in the maximum micturition pressure compensates for the increased outflow resistance and there is often no reduction in the free flow rate until a later stage when the classical picture

TABLE 1.1

The prevalence of detrusor instability demonstrated urodynamically in patients with prostate obstruction (after Abrams 1985).

References	No of Pts.	% Detrusor Instability.	Text refers to:-
*Leppanen 1962	43	56	"Hypertonic"
*Makrigiannis 1972	50	56	Detrusor Instability
Anderson 1982	93	49	"Hyperreflexia"
*Abrams 1977	203	63	Detrusor Instability
Price 1980	40	72	Detrusor Instability
Meyhoff 1984	60	80	Detrusor Instability
Coolsaet 1984	139	52	Detrusor Instability
*Frimodt Moller 1984	84	65	Detrusor Instability

* These series may contain obstructed patients as no definition of obstruction in relation to instability was presented.

TABLE 1.2

The incidence of detrusor instability before and after prostatectomy (after Abrams 1985).

References	Pre-operative %	Post-operative %
Leppanen 1962	80 after RPP 35 after TURP	0 22
Makrigiannis 1972	34 after RPP 50 after TURP	0 25
Anderson 1982	49	31
Abrams 1977	56	23
Price 1980	72	28
Meyhoff 1984	81 after RPP 79 after TURP	55 44
Frimodt Moller 1984	65	22

TURP = Transurethral resection of the prostate
RPP = Retropubic prostatectomy

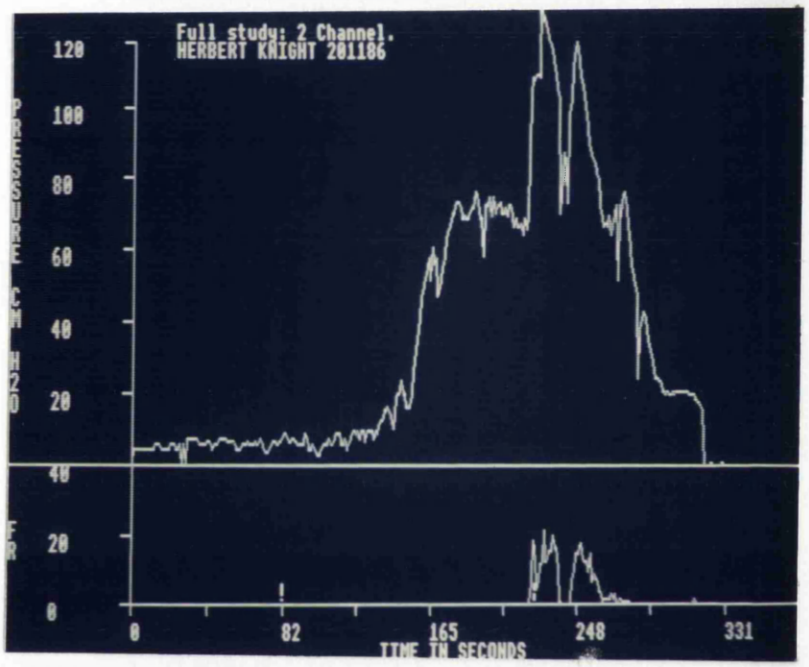
of high pressure/low flow is seen (Figure 1.4).

Irritative symptoms could arise from either motor or sensory dysfunction (Bates, 1971b), but are usually the symptomatic manifestation of secondary detrusor instability. Indeed symptoms associated with detrusor instability are often the most troublesome to the patient and usually provide the stimulus prompting medical referral. Although effective relief of bladder outflow obstruction usually leads to complete resolution of associated detrusor instability within six months, such symptoms may persist for up to one year following relief of obstruction. When detrusor instability persists following surgery it commonly results in troublesome symptoms. Studies of patients with post-prostatectomy incontinence reveal a significant association with persisting detrusor instability. Fitzpatrick et al. 1979 studied 68 patients; 45 had persisting instability associated with post-prostatectomy incontinence, 37 had no urodynamic evidence of residual obstruction.

The causal association between instability and prostatic obstruction has recently been challenged by Abrams (1985), who notes that other types of outflow obstruction are not associated with a similar predisposition to detrusor instability. In addition, the incidence of detrusor instability increases with advancing age in the population, even in unobstructed patients. Andersen (1982) showed detrusor hyperreflexia in 53% of 17 asymptomatic elderly males aged 60-75 and

Figure 1.4

Cystometrogram trace demonstrating unstable prostatic obstruction, *=detrusor instability towards the end of filling, +=maximum voiding detrusor pressure. note the maximum flow rate of 20 ml/sec which by itself is not indicative of bladder outflow obstruction.



in addition, he confirmed the poor correlation between detrusor instability and "severity of obstruction" as judged by urodynamic parameters, which had previously been reported by Arnold (1973).

Although the possibility of a causal link between detrusor instability and outflow obstruction is contentious, it is clear from animal studies that bladder outflow obstruction does lead to important functional changes within the bladder (Sibley 1987). The resultant reversible changes in detrusor function appear to provide a good model for the secondary detrusor instability seen in the human. The literature on this subject is reviewed in detail in Chapter 4.

It is therefore evident that there is an association between bladder outlet obstruction and detrusor instability which resolves in up to two thirds of patients following surgery, and is an important cause of troublesome symptoms. There have been few reported studies of the combined morphological and physiological changes which occur in the bladder in response to obstruction and which may contribute to disordered detrusor function. It was one of the primary intentions of the work presented in this thesis to investigate this matter further using a combination of histochemical and pharmacological techniques to study the effect of outflow obstruction on the innervation and function of the human detrusor (Chapters 5-6).

1.4 The neural control of the human prostate

Neural Influences and Benign Prostatic Hyperplasia.

Benign prostatic hyperplasia is an almost universal finding in men with normal gonadal function, from the 5th decade of life. Prostatic enlargement causes urethral compression and results in an increased resistance to the bladder outflow tract. The incidence of prostatic obstruction increases with age from the 6th decade of life onwards; but the onset of symptoms is gradual and usually only slowly progressive (Ball et al. 1981). However, it has been estimated that a 40-year-old man has a 29% risk of undergoing a prostatectomy during his lifetime (Glynn et al. 1985)

The traditional view of the pathogenesis of prostatic obstruction regards simple mechanical urethral compression, resulting from an increase in prostatic bulk, as being the major component. In recent years this view has been challenged. It is now well recognised that the degree of prostatic enlargement as assessed clinically correlates poorly with the severity of outflow obstruction (Turner-Warwick, 1973). Whilst this can be attributed solely to the asymmetric nature of prostatic enlargement (Hunter 1786) an additional factor which has been increasingly recognised in recent years is the important contribution provided by the neural control of the prostate to the genesis of bladder outflow obstruction (Donker et al. 1972; Furuya et al. 1982).

A substantial body of evidence now exists to support the hypothesis that the sympathetic nervous system controls the contraction of the prostatic musculature via the release of noradrenaline which binds onto adrenoceptors within prostatic muscle. This has allowed the development of non-surgical pharmacological treatment of benign prostatic outflow obstruction using sympathetic α adrenoceptor blockade (Caine 1986 a,b).

Despite the undoubted importance of the prostate gland in both health and disease, surprisingly little information is available relating to the ultrastructure of its innervation. Recent reports have suggested that sensory nerve stimulation within the prostate gland may be important in the aetiology of the associated secondary detrusor instability (Chalfin and Bradley 1982). However, few previous studies have documented the motor innervation and none has described the sensory innervation of the human prostate. Although the preeminence of the sympathetic innervation in motor control is well established there is unresolved controversy over the mechanism by which this acts and the precise roles of α_1 and α_2 adrenoceptors. A review of the literature relating to the anatomy and innervation of the human prostate is presented in Chapter 7.

Chapter 8 of this thesis contains the results of an histological study of the innervation of the normal and hyperplastic human prostate. Chapters 9 and 10 present the results of experimental

studies using pharmacological, biochemical and histochemical techniques to document the mechanism by which the sympathetic nervous system stimulates contraction of prostatic musculature.

CHAPTER 2

THE ANATOMY AND INNERVATION OF THE BLADDER

2.1 INTRODUCTION

The urinary tract provides a highly sophisticated system of conduits, which allows the conversion of a continuous and involuntary production of urine by the kidneys into the intermittent, consciously controlled voiding of urine (micturition) in appropriate circumstances. It is also designed to protect the nephrons of the kidney from damage by the retrograde transmission of pressure or infection from the bladder.

The urinary bladder has two main functions:- the collection and low pressure storage of urine and its subsequent expulsion at an appropriate time and place. Disruption of the normal action of the bladder usually produces significant clinical symptoms. Such symptoms may be the consequence of either local pathological conditions affecting the bladder and its outflow tract or disordered neural control of detrusor muscle. In order to understand the clinical consequences of disordered bladder function, it is necessary first to appreciate the structure of the bladder, its innervation and neurophysiological control.

2.2 HISTORICAL ASPECTS

The first recorded reference to the human bladder is to be found in the ancient Indian literature - in the "Susmata Samhita" (Mettler 1947). Fallopius is accredited with being the first to recognise that the bladder is

not an inert reservoir, but is emptied by active contraction of its muscle coat. A later account attributable to Galen (2nd century AD) described the walls of this organ to be comprised of fibres arranged in discrete functional layers (Hald 1969). This concept was considered to be correct until the early part of this century (McCrea 1926). Although Spiegel initially coined the term musculus detrusor urinae (detrudare = to drive out), and applied it to the longitudinal muscle coat alone (Griffiths 1891), more recent studies have demonstrated that individual muscle fibres traverse all layers of the bladder wall (Hunter 1954, Woodburne 1960).

2.3 ANATOMY

The human urinary bladder is an organ of variable size and shape as dictated by the volume of fluid present within its lumen. It has three distinct histological layers, an outer adventitial connective tissue layer, a middle smooth muscle coat and an innermost mucous membrane of transitional cell epithelium. This mucus membrane is supported by lamina propria and muscularis mucosa (Dixon 1983) and provides an elastic lining which is impervious to urine.

The mammalian bladder can be subdivided into three distinct regions on the basis of embryological, histological and functional criteria.

a) bladder body.

b) trigone.

c) bladder neck.

The detrusor muscle is responsible for the normal motor function of the

bladder and although it can be further subdivided into three concentric ill-defined layers it is best considered as a single unit comprising interlocking muscle fibres which combine to form a functional syncytium. The detrusor muscle is under the control of the autonomic nervous system and receives a rich innervation comprising three main groups of nerves which form dense plexuses amongst the smooth muscle cells. Within the bladder, nerves pursue a tortuous course, which provides slack that can be taken up during bladder distension (Fletcher and Bradley, 1969).

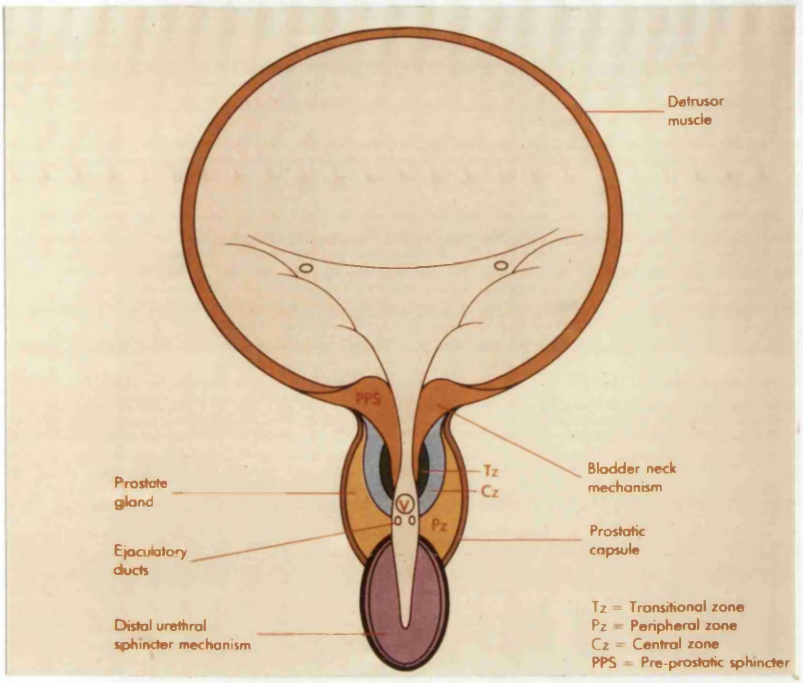
In the male there are two important sphincteric mechanisms (Figure 2.1 a&b), a proximal "bladder neck mechanism" and a urethral sphincteric mechanism lying at the apex of the prostate (the "distal sphincter mechanism").

The male bladder neck subserves two functions. It is a powerful urinary sphincter and its contraction during ejaculation is essential to the prevention of the retrograde transmission of semen into the bladder. In addition, there is a more distal urethral sphincteric mechanism lying at the apex of the prostate. This distal sphincteric mechanism is extremely powerful as evidenced by its ability to maintain continence even when the bladder neck has been rendered totally incompetent by bladder neck incision or prostatectomy. The urethral sphincter mechanism is comprised of intrinsic urethral smooth muscle and extrinsic striated muscle components.

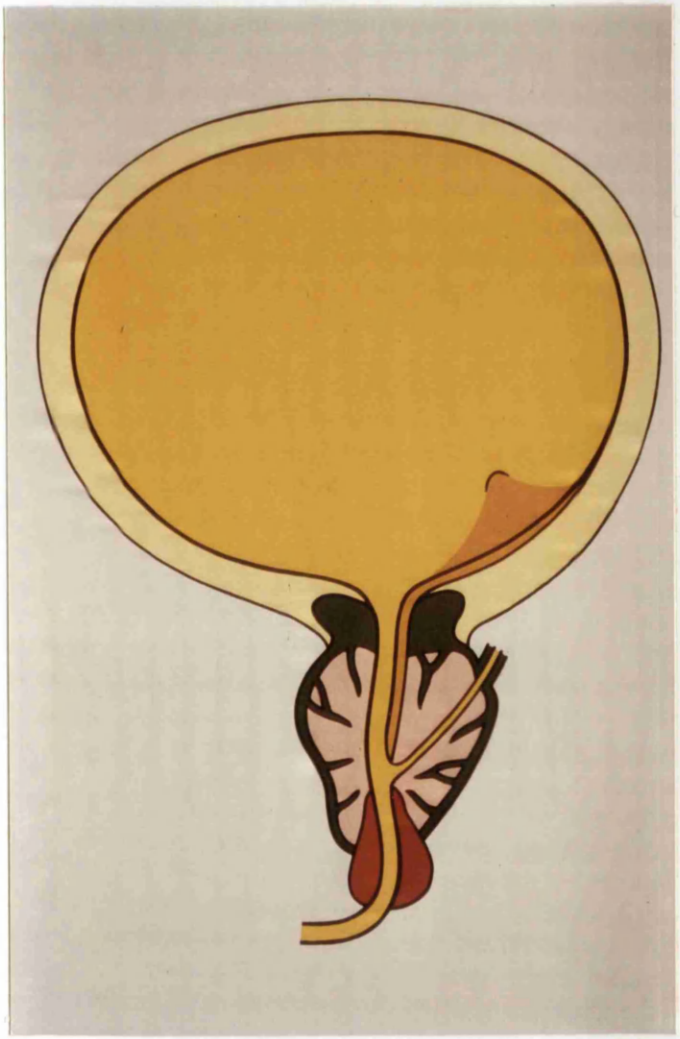
Although bladder neck and urethral smooth muscle receive a dual autonomic innervation, the sympathetic nervous system is thought to be of

FIGURE 2.1

a) Diagrammatic representation of the male lower urinary tract.



b) The male lower urinary tract in sagittal section.



principal importance in the male. The efferent innervation of the striated muscle (the extrinsic component of the urethral sphincter), arises predominantly from cell bodies lying in a specific area of the sacral anterior horn known as Onufs nucleus (Onufrowicz 1900). A number of aspects of the innervation of this sphincter are controversial, for example, whether the somatic nerve fibres pass via the pudendal nerve (Vodusek 1983) or the pelvic splanchnic nerves (Gosling, Dixon et al 1983) and whether there is a significant autonomic innervation.

The trigone is a triangular area framed by the ureteric orifices above and the internal urethral meatus below. The majority of the muscle cells comprising the trigonal detrusor muscle are histologically indistinguishable from their counterparts in the remainder of the bladder. A thin superficial muscle layer is thickened superiorly to form a prominent slightly curved ridge known as the superficial trigonal muscle. This is comprised of small diameter muscle cells which are morphologically distinct from the detrusor muscle (Gosling, Dixon et al. 1983); they arise from mesodermal structures and hence have a different embryological origin to the bladder. Despite its rather insignificant mass, it is suggested that by contracting during micturition, this muscle occludes the ureteric orifices to prevent vesico-ureteric reflux (Hutch 1963, Tanagho and Pugh 1963).

2.4 INNERVATION

In mammalian species the urinary bladder receives an innervation from the parasympathetic and sympathetic divisions of the autonomic nervous

system which traditionally contain the neurotransmitters acetylcholine and noradrenaline respectively (Langley and Anderson 1895a, Elliott 1907), although recently, a number of non-adrenergic, non-cholinergic (NANC) neurotransmitters have been identified (Ambache and Zar 1970). In the human bladder, parasympathetic stimulation initiates bladder contraction (Learmonth 1931) via the release of the transmitter acetylcholine. At present, the role of adrenergic sympathetic neurons and NANC sensorimotor nerves containing putative peptidergic, amine and purinergic neurotransmitters is still the subject of debate. The remainder of this chapter reviews the anatomy and ultrastructure of bladder innervation and its potential neurophysiological role.

Mobley and co-workers (1966) were the first to report that the body and the base of the human bladder have a dual parasympathetic and sympathetic innervation which is distributed both in company with and separately from blood vessels. However it has been recognised for a long time that there is a dual autonomic innervation to the bladder in most mammalian species (Langley and Anderson 1895a, Elliott 1907). The subsequent literature contains a number of conflicting observations, which can be related to the extreme heterogeneity of the material studied by different authors, occasioned by the difficulties inherent in obtaining "normal" human tissue.

A review of the literature reveals the following as being the principal sources of error :-

1. Differences in the age and sex of the subjects studied.

2. The co-existence of unrecognised disease.

3. Difficulties in standardising the site and type of tissue samples collected.

4. The diversity and limitations of the investigative techniques used.

2.4.1 MOTOR INNERVATION

Parasympathetic Neurons

Considerable methodological problems are associated with the specific histochemical localisation of peripheral acetylcholine-containing neurons. Current techniques rely on the relatively non-specific technique first described by Koelle and Friedenwald (1949), which demonstrates the presence of the ubiquitous enzyme acetylcholinesterase (AChE). Indeed, it has been suggested that much of the AChE activity which can be demonstrated in detrusor muscle is related to non-specific staining of the muscle cell membrane (Raezer et al 1973). A more specific technique for the histochemical demonstration of acetylcholine-containing nerve fibres depends upon the visualisation of ChAT (choline acetyl transferase), the acetylcholine synthesising enzyme (Burt and Silver 1973). This technique is unfortunately not suitable for use in the demonstration of peripheral nerves, since it only stains neuronal cell bodies and not terminals (Alm 1978). Despite the problems outlined above it is now generally considered that AChE staining does provide reliable visualisation of peripheral cholinergic neurons (Elbadawi 1982), although it must be remembered that it will also non-specifically stain nerves containing a variety of other neurotransmitters.

Cholinergic nerves identified by their AChE content (Gosling and Dixon et al 1983) are the principal neuronal population within the detrusor body. These AChE-positive neurons form a net-like plexus in all areas of the bladder body with large AChE positive nerve trunks clearly identifiable in the basal layers of smooth muscle cells (Ek et al 1977). Electron microscopy has shown these neurons to lie in close apposition to muscle cells (Taira 1972), where they are likely to mediate the major control of detrusor smooth muscle via the release of acetylcholine.

Sympathetic neurons

Techniques to specifically identify noradrenergic nerves demonstrate that in contrast to the rich parasympathetic nervous supply the sympathetic supply to the bladder body is sparse and non-uniformly distributed. In the normal bladder adrenergic terminals can be identified predominantly in association with blood vessels rather than smooth muscle and are thought to be important in the control of vasculature (Elbadawi and Schenk 1966, Sundin et al 1977).

The trigone can be subdivided anatomically into two areas as noted above, each of which has a distinctive neural innervation. The deep layer is identical in terms of structure and innervation to the main detrusor muscle - the trigonal detrusor muscle. In contrast, the superficial layer of the trigone possesses few AChE-positive nerves but a predominance of noradrenergic sympathetic neurons (Gosling, Dixon et al. 1983, Ek et al. 1977).

Benson et al. (1979) demonstrated adrenergic nerve terminals using a fluorescent glyoxylic acid technique; the distribution was patchy within muscle bundles, but there was an association between these nerve fibres and blood vessels. No sex-related difference in the innervation of the bladder wall was evident, but they described an age-related decline in adrenergic innervation, a similar phenomenon to that which had previously been noted in human sympathetic ganglia (Hervonen et al. 1978). Nordling et al. (1983) reported a sparse distribution of noradrenergic nerve terminals both within the detrusor and the trigonal smooth muscle.

The differences reported by various investigators in the morphology and distribution of adrenergic nerves within the human bladder may be more apparent than real. Catecholamines are labile and histochemical results are notoriously subject to methodological errors arising from the collection of tissue and its subsequent handling.

Electron microscopy (vide infra) confirms that in the bladder dome the majority of nerve fibres are likely to be cholinergic whilst, in the trigone, adrenergic endings still represent only a small proportion of the total (Dixon, Gosling et al. 1987). Such work confirms the results of light microscopy studies and in particular supports the use of the non-specific AChE technique. As elsewhere in the body, it is likely that the sympathetic and parasympathetic nerve supplies to the bladder have opposing actions. Clearly, in view of the sparse distribution of innervation, sympathetically mediated inhibition of bladder smooth muscle activity is unlikely to depend

upon a direct action on detrusor musculature.

Sympathetic/Parasympathetic interaction

Evidence from animal studies suggests that detrusor inhibition is mediated by sympathetic neurons acting via α_1 receptors located upon parasympathetic cell bodies within peri-vesical or pelvic plexuses and acting via the presynaptic inhibition of acetylcholine release (deGroat and Booth, 1980). A plausible hypothesis is that vesical ganglia act as filters on the efferent pathways to the bladder, functioning as blocks when pre-ganglionic firing is low, but conversely facilitating neurotransmission when activity is increased. Although numerous ganglia each containing up to twenty neuronal cell bodies can be identified within the human bladder wall, these appear to contain cholinergic neurons with no evidence of noradrenergic terminals within them. In view of the absence of associated adrenergic nerve terminals it therefore seems unlikely that there is a significant interaction between cholinergic parasympathetic and sympathetic autonomic neurons at the level of the bladder ganglia (Dixon et al. 1983; Gilpin et al. 1983). However, in both the perivesical and pelvic plexuses there are numerous synaptic contacts between the constituent sympathetic and parasympathetic neurons (Dixon and Gilpin et al. 1983).

Non-adrenergic, non-cholinergic innervation

A third population of neurons found within the bladder comprises the so-called non-adrenergic, non-cholinergic (NANC) sensorimotor nerves (Mundy 1984). These nerves contain a number of putative neurotransmitters, which

can be identified by the use of immunofluorescence techniques (Ambache and Aboo Zar, 1970). The existence of peripheral nerves that conformed to neither of the traditional autonomic groups was first suggested in the 1960's (Burnstock 1969). The original reason for the interest in this third group of NANC nerves was to find an explanation for the so-called atropine resistance found in studies of the animal bladder (Ambache and Aboo Zar 1970).

In electron microscopy studies, differentiation between cholinergic and adrenergic nerve terminals depends on identification of the type of transmitter vesicles present. Presumptive cholinergic terminals contain small agranular vesicles whilst adrenergic endings contain small dense-core vesicles. In both neural populations large granular vesicles which are thought to contain peptides may also be found (Larsson 1977). More recently, a precise correlation between morphological appearance and the contents of vesicles has been challenged (Daniel et al. 1983). Nevertheless, in recent years a considerable body of histochemical and biochemical evidence has accumulated which indicates that peptide neurotransmitters are widely distributed in structures involved in the regulation of bladder and urethral function by both central and peripheral nervous systems (Maggi and Meli 1986, deGroat and Kawatani 1985).

Unfortunately, despite the abundance of research there have been few attempts to integrate structural and functional observations. An important contributory factor in the case of the human bladder is the paucity of

physiological and pharmacological data on the functional role of these putative neurotransmitters. If the classical criteria required to identify a putative neurotransmitter are strictly applied, few if any of these putative peptide neurotransmitters would have an established role.

Criteria for classical neurotransmitters.

1. Synthesis and storage in nerve terminals.
2. Calcium-dependent release on nerve stimulation.
3. Occupation of specific receptors on the post-junctional cell leading to changes in its activity.
4. Inactivation by enzyme and/or by uptake.
5. Drugs producing parallel block (or potentiation) of the responses to neural stimulation and the exogenously applied substance.

In an attempt to integrate and explain the role of these NANC substances it has been suggested that some neurons may store and release more than one transmitter. This hypothesis is based on the work of Burn and Rand (1965) and comparative studies of the evolution of the autonomic nervous system (Burnstock 1969). There is now considerable support for the intraneuronal coexistence of peptides and purine nucleotides with classical neurotransmitters such as acetylcholine and noradrenaline outside the urinary tract (Hokfelt et al 1980, Burnstock 1986, Gu et al. 1984). Although the functional interaction of these putative peptide neurotransmitters within the classical autonomic innervation remains to be established, possible roles include:- co-transmission, neuro-modulation and a trophic function.

In the early 1970's a large number of compounds (including peptides, purines, monoamines and amino acids were screened for a potential role as NANC neurotransmitters within the bladder. A substance that satisfied the classical criteria (see above) was the purine nucleotide adenosine 5-triphosphate (ATP). Nerves containing this were termed "purinergic" (Burnstock 1972). Histological attempts to identify purinergic nerves have so far been disappointing, but on electron microscopy it has been reported that purinergic nerve terminals contain granulated vesicles larger than those within cholinergic or adrenergic endings, so called "large opaque vesicles" (Burnstock 1972). There is well documented evidence of an important functional role for purinergic nerves in non-primate mammals, in particular the guinea pig (Burnstock et al. 1978; Mackenzie and Burnstock 1984); but this subject is still the matter of some debate (Ambache et al. 1977; Levin et al 1986). Recently a number of peptides including vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), substance P (SP), somatostatin, calcitonin gene related peptide (CGRP) and enkephalin have been identified in the bladder innervation in experimental animals and have been ascribed a neurotransmitter or neuromodulatory role (Maggi and Meli 1986). Very little histochemical work on the human bladder has been reported, but the presence of VIP, NPY, substance P and somatostatin has been confirmed (Gu et al. 1984).

The role of the NANC neurotransmitters in the human bladder is as yet poorly understood. Although substance P and CGRP are thought to be

involved in afferent nerve pathways (Maggi and Meli 1986, Mundy 1984), VIP is the only peptide transmitter for which any defined physiological role has been identified. In the cat 10-15% of bladder ganglia exhibit VIP immunoreactivity (Kawatani et al 1985). VIP has also been detected in intramural ganglia of the human urinary bladder and in nerve fibres which are particularly densely distributed beneath the epithelium, around blood vessels and in the muscle layers. VIP is present in higher concentration in the trigone than in the dome of the bladder (Polak and Bloom 1984; Gu et al 1984).

It has been reported that VIP tissue levels are markedly reduced in the bladder of patients with idiopathic detrusor instability (Gu et al. 1983a). The myogenic (tetrodotoxin-resistant) contractile activity of the human detrusor, which is inhibited by exogenous VIP is greater than normal in detrusor hyperreflexia as contrasted to that of urodynamically normal bladders (Kinder et al. 1985). A plausible hypothesis which explains these observations is that detrusor instability results from a disorder of intrinsic inhibitory mechanisms, involving endogenous VIP-ergic fibres within the detrusor.

2.4.2 SENSORY INNERVATION

Whilst there are still marked gaps in our knowledge of the efferent (motor) innervation of the lower urinary tract, even less is understood about afferent innervation. A major factor which has hindered further understanding of this aspect of bladder innervation is the difficulty in

correlating structure and in vitro pharmacological results with in vivo sensory function. When considering sensation within the lower urinary tract it is important to regard the bladder, posterior urethra and trigone as a combined functional complex.

Although complex sensory nerve endings (Kleyntjens and Langworthy 1937) and identifiable Pacinian corpuscles (Fletcher and Bradley 1970, Feher et al. 1982) have been identified in animal bladders, no distinct anatomical sensory receptors have yet been identified in man. It seems probable that sensory information arises from stretch receptors in the detrusor muscle (Iggo 1955), and it appears that in the cat the majority of bladder afferents respond to tension changes within detrusor musculature (Winter 1971). In man, stimulating the mucosa of the urinary bladder with a needle (Moore 1924) or using electrodes (Frimodt-Moller 1972) does produce pain.

A fine plexus of acetyl-cholinesterase positive nerve fibres can be demonstrated within the lamina propria of the urethra and bladder. This is especially dense beneath the epithelial lining and these nerves are particularly prominent at the base of the bladder, around the trigone and bladder neck (George and Dixon 1986). It has been suggested that these acetylcholinesterase positive nerves which contain numerous agranular synaptic type vesicles are visceral afferent nerve endings (Gosling and Dixon 1974). The principal basis for this hypothesis is a lack of recognisable target sites with no apparent correlation of innervation with a demonstrable effector role. This hypothesis has been supported by observations in patients

with cholinergic dysautonomia (Kirby 1987); it was noted that despite a quantifiable reduction in the density of cholinergic nerves in the bladder muscle with a corresponding motor deficit, the sub-epithelial plexus was unaffected and bladder sensation was preserved. These conclusions are however challenged on the basis of animal studies, for example, observation of neuronal degeneration following surgical ablation of spinal ganglia documented that there was a degeneration rate of less than 1% for submucosal axons with agranular synaptic vesicles (Uemura et al. 1975). In recent years substance P-like immunoreactivity has been reported to occur within isolated nerve fibres in the lamina propria and subepithelial nerves (Gu et al. 1984, Alm et al. 1979). This peptide had previously been proposed as a sensory neurotransmitter (Nicoll et al. 1980). More recently CGRP has been shown to coexist within the same group of neurons (Yokokawa at al. 1986).

The density of innervation observed on ultrastructural study is supported by clinical experience, stimuli affecting the base of the bladder and trigone producing the most marked symptoms (eg. strangury). Conversely, inflamed mucosa around the bladder dome is usually experienced by the patient as a dull suprapubic ache. It has been suggested that sensory perception within the bladder depends upon both extroceptive and proprioceptive receptors (Hald 1969), indeed, proprioceptive receptors discharge at a rate dependent on the speed of bladder filling (Klevmark 1974).

Frimodt-Moller (1972) reported a method of evaluating bladder sensation, the technique of mucosal electrosensitivity. A stimulator delivered via a silver wire a constant square wave impulse, the amplitude of which could be altered, the lowest amplitude producing a sensation recorded as the electrical perception threshold. Subsequent modifications have been made (Kiesswetter 1977, Powell and Feneley 1980), but this technique remains crude and of limited usefulness.

2.5 SUMMARY

Morphological evidence indicates that the human bladder receives a dense AChE-positive innervation predominantly comprised of acetylcholine containing parasympathetic neurons. There is limited evidence for a significant adrenergic sympathetic innervation to all except the base of the bladder (trigone, bladder neck). A number of nerves containing putative neurotransmitters have been identified, but their role in the human bladder is at present poorly substantiated. Ultrastructural studies have defined the occurrence and distribution of different neuronal populations, characterised by their content of neurotransmitter granules. Overall these results contribute little in isolation and their functional significance must be evaluated by the concurrent use of physiological and pharmacological studies. The central nervous system control of bladder innervation and its associated pharmacological mechanisms are reviewed in Chapter 3.

CHAPTER 3

THE PHYSIOLOGY OF MICTURITION

Before considering the investigation of disorders of micturition it is first essential to analyse the neural mechanisms which control bladder function. Bladder function can be subdivided into two interrelated yet distinct phases, urine storage and its controlled voiding at an appropriate time and place. Most contemporary knowledge is based on studies with experimental animals. Although it can be difficult and is often misleading to relate findings from animal models to man, such information is essential since human data on the central nervous control of the bladder can only be derived from clearly defined clinical syndromes and isolated spinal cord lesions.

3.1 NEUROPHYSIOLOGICAL CONTROL OF THE BLADDER

3.1.1 Local neural pathways

The spinal segments S2-S4 acting via efferent parasympathetic cholinergic neurons are responsible for the initiation and maintenance of detrusor contraction. Damage to these spinal segments results in abolition of the micturition reflex in man (Denny Brown and Robertson, 1933). After leaving the sacral foramina the pelvic splanchnic nerves, containing the parasympathetic innervation to the bladder and possibly some efferent somatic neurons to the intrinsic component of the urethral sphincter, pass lateral to the rectum to enter the inferior hypogastric or pelvic plexus. They

are joined by the hypogastric nerve containing efferent sympathetic nerve fibres originating from the lower three thoracic and upper two lumbar segments of the spinal cord (Warwick and Williams 1973). When combined they form a plexus lying at the base of the bladder. The limited knowledge available suggests that the pudendal nerve transmits urethral mucosal sensation (Nathan 1956) and it has long been suggested that the afferent pathway of the micturition reflex is carried via the pelvic nerves (Learmonth 1931). Additional afferent information is likely to be transmitted from the trigone via sympathetic neuronal pathways in the hypogastric nerves (Winter 1971). From observation of patients undergoing anterolateral cordotomy Nathan and Smith (1951) concluded that some bladder and urethral sensation in the afferent limb of the micturition reflex passed proximally via the spinothalamic tracts.

3.1.2 Reflexes governing micturition

Barrington initially described five reflexes associated with micturition in the cat (1914), and added a further two on the basis of further study (1931, 1941). Two of these reflexes had their reflex centres in supraspinal sites (medulla and pons) and caused strong and sustained contractions. He considered these as essential for normal micturition, since bladder contraction and urethral relaxation were not coordinated after experimentally produced high spinal transection. The remaining five reflexes appeared confined to the spinal cord.

Although it is tempting to relate these findings to man, Denny-Brown

and Robertson (1933a) failed to detect either initiation of micturition or vesical contraction resulting from distension of the posterior urethra in man and concluded that micturition was a reflex act resulting from bladder distension and mediated by a centre in the sacral cord (Denny-Brown and Robertson 1933b). More recently, Kuru (1965) has proposed that many inter-related reflexes act upon the sacral micturition centre, exerting both excitatory and inhibitory effects.

Urine storage

During bladder filling, afferent activity from stretch receptors increases and passes via the posterior roots of the sacral cord and the lateral spinothalamic tracts to the brain, thereby mediating the desire to void. Activity within the striated component of the urethral sphincter is increased, and local spinal reflex activity in turn stimulates the pudendal motor neurons of the nucleus of Onufrowicz, which enhances the activity within striated muscles of the pelvic floor and sphincter.

Local factors are important during bladder filling and these include not only receptive relaxation (Tang and Ruch 1955), but also the passive visco-elastic properties of the bladder wall. Both abnormal bladder morphology resulting from collagenous infiltration, hypertrophy or altered muscle structure (e.g. obstructed bladder) and abnormal detrusor smooth muscle behaviour, either primary or secondary to neural dysfunction, could contribute to the genesis of poor bladder compliance and detrusor instability.

Initiation of micturition

Once a threshold level of filling has been achieved, which will depend on circumstances and vary considerably between individuals, the increasing afferent activity impinges upon consciousness, and the subject becomes aware that the bladder is filling. Except during infancy, the normal human has complete volitional control over these reflex pathways.

When micturition is initiated by the cerebral cortex a complex series of bladder/brain stem reflexes are involved (Kuru 1965). Urethral relaxation precedes detrusor contraction (Tanagho and Miller 1970), there is a simultaneous relaxation of the pelvic floor muscles (Porter 1962) and these events are accompanied by funnelling of the bladder neck (Lund et al 1957). The inhibitory activity of the higher centres on the sacral centres is lifted, allowing parasympathetically controlled detrusor contraction to occur with a corresponding relaxation of the urethra /prostate/ bladder neck complex resulting from reciprocal sympathetic nerve inhibition. In addition to these primary actions, other important secondary events include contraction of the diaphragm and anterior abdominal wall muscles, and the specific behavioural changes associated with voiding.

At the end of voiding the proximal urethra is closed in a retrograde fashion, the "milkback" seen at videocystometry. Once these events are completed inhibition is reapplied to the sacral centres by the cortex and the next filling cycle starts.

3.2 PHARMACOLOGICAL RESPONSES OF THE BLADDER

Animal experimentation has been helpful in clarifying the complex local neural interactions which participate in the control of lower urinary tract function. It is however important to take care in interpreting such data and extrapolating results from animals to man, since there are important inherent species differences. The following examples demonstrate this:- physiological adaptations to encompass behavioural characteristics such as territorial marking; pharmacological differences with a high proportion of non-adrenergic, non-cholinergic neurotransmission in the control of detrusor function in rodents; anatomical differences, in particular, the upright position of the human and the extra-peritoneal position of the bladder base and urethra which completely change the influence of supporting tissues.

The traditional approach has been to subdivide the autonomic nervous system into two divisions, sympathetic and parasympathetic, based on the two neurotransmitter substances, noradrenaline and acetylcholine respectively. Bearing in mind the caveat expressed above it must be remembered that most of our knowledge of human bladder neurophysiology is derived from animal studies. Furthermore, in recent years, the recognition of new putative transmitter substances has complicated an already controversial area. It is the intention of this chapter to summarise the current literature on this subject.

3.2.1 The role of the parasympathetic nervous system.

Coordinated contraction of the urinary bladder at the time of micturition

is initiated and maintained by parasympathetic nervous stimulation via the pelvic nerves in all mammalian species studied (Andersson and Sjogren 1982). The early recognition of cholinergic transmission at many synapses resulted in acceptance of acetylcholine as the post-ganglionic transmitter in the bladder and formulation of the theory of cholinergic transmission (Dale 1933). This hypothesis is in accordance with the histological evidence of a dense uniform network of cholinergic (acetylcholinesterase-positive) fibres and the corollary that all parts of the bladder, including the trigone and proximal urethra, contract when exposed to acetylcholine, an effect inhibited by atropine (Todd and Mack 1969, Nergardh 1975).

Further evidence in support of acetylcholine being an excitatory neurotransmitter is provided by the work of Carpenter and Rand (1965) who studied whole bladder preparations from the rat and assayed the bath medium for acetylcholine both at rest and during nerve-mediated electrical stimulation. Acetylcholine output was increased 150-fold during electrical stimulation. These observations are supported by in vivo studies in both rhesus monkeys (Craggs and Stephenson 1985) and man (Cullumbine et al. 1955).

Work utilising receptor binding techniques with 1-quinuclidinyl (phenyl4-^{3}H) benzilate (^{3}HQNB) to visualise cholinergic muscarinic receptor binding sites confirms that the human detrusor muscle contains a number of such receptors (Levin et al. 1982). Ligand binding data using the agonist carbachol suggests that there is more than one class of these muscarinic

receptors (Nilvebrant et al. 1985), leading to the tempting speculation that in the future it might be possible to develop drugs with precise selectivity for muscarinic receptors in the human bladder.

Partial atropine resistance of bladder contractions was first noted by Langley and Anderson (1895) during electrical stimulation of the parasympathetic nerve supply to the human bladder. Refractoriness to atropine was also documented by Henderson and Roepke (1934, 1935), in experiments using pelvic nerve stimulation in dogs. Despite further confirmation of this phenomenon in the cat bladder (Edge 1955) and several other parasympathetically innervated organs, "the nature of parasympathetic post-ganglionic neurons in the bladder remain(ed) unsolved" (Ambache 1955).

Vanov (1965) reported that atropine resistance noted during his experiments on the rat bladder was counteracted by a disruption of acetylcholine synthesis using hemicholinium; on this basis it was suggested that the phenomenon of atropine resistance was produced by a local build up of endogenous acetylcholine around the post-synaptic membrane, sufficient to antagonise receptor blockade by atropine. Other explanations which have been suggested to explain the presence of non-cholinergic transmission have included:

a) that muscarinic acetylcholine receptors in the detrusor muscle behave anomalously towards atropine.

b) that neurogenic acetylcholine is released in close proximity to receptors and hence beyond the atropine - barrier (Dale and Gaddum 1930).

Contemporary knowledge finds little evidence to support these hypotheses and in particular, the Dale/Gaddum hypothesis has further been discredited by Dumsday (1971) who calculated that the synaptic volume was sufficient to allow access of atropine to receptors.

Ambache and Zar (1970) taking note of these suggestions used much lower electrical stimulation to avoid flooding the receptors with neurotransmitter and provided clear evidence in animal studies, predominantly on guinea pigs but also in cats and rabbits, that atropine-resistant neurotransmission was a definite entity and appeared to be mediated via a truly non-cholinergic mechanism. They suggested that adrenergic nerves were unlikely to be implicated in non-cholinergic neurotransmission in the guinea pig, since administration of noradrenaline produced detrusor relaxation and neural stimulation was unaffected by both α and β adrenoceptor blockade. This has been confirmed by work showing that depletion of catecholamines from the bladder with reserpine was without effect (Dean and Downie 1978).

The concept of non-adrenergic, non-cholinergic neurotransmission (NANC) is now widely accepted (Taira 1972, Andersson and Mattiasson 1982) and the presence of marked interspecies variation in NANC bladder control is well recognised. The nature and indeed role of NANC neurotransmission is still the subject of debate, a situation further complicated by the likelihood that there are a number of different neurotransmitter substances involved (Burnstock 1986).

Hindmarsh et al (1977) reported that electrically induced contraction of human detrusor muscle strips was only partially sensitive to atropine, thus lending support to the view that there might be a NANC component. Eaton and Bates (1982), reporting an in vitro pharmacological study investigating samples of both normal and unstable obstructed detrusor, noted only "partial inhibition" by atropine in both groups.

Cowan and Daniel (1983) in a study of normal female human detrusor suggested that there might be a tetrodotoxin(TTX)-resistant NANC excitatory system, representing approximately 50% of the contractile response to short-pulse electrical field stimulation. These findings are controversial, since their interpretation relies upon the postulate that there is stimulation of a TTX resistant non-muscle site, whereby these nerves do not release mediator by the standard mechanism involving sodium conductance.

In contrast, Sjogren and his co-workers (1982) found that detrusor strips from 33 patients deemed to have normal bladders (although urodynamic investigation had not been carried out), invariably demonstrated a response to transmural electrical stimulation which was almost completely inhibited by atropine (95%). Interestingly, in the same study muscle strips from patients with prostatic outflow obstruction exhibited atropine resistance of up to 50%.

Kinder and Mundy (1985b), reported a study of 23 detrusor muscle strips obtained from 13 urodynamically normal patients and documented a 92.7% inhibition by atropine of the response to nerve-mediated stimulation.

Similarly, Sibley (1984) concluded that nerve-mediated activity in normal human bladder is exclusively cholinergic, as contrasted to a significant atropine-resistant component in the rabbit (58%) and pig (22%). In the same comparative study bladder strips from patients undergoing prostatectomy were also studied, and although an atropine-resistant component of 20% was recorded, this was also TTX resistant and therefore unlikely to be nerve-mediated.

The apparent conflict of data which exists in the literature can be explained by heterogeneity of the tissue investigated and variation in experimental procedures. It has to be concluded that since the normal human detrusor does not possess significant atropine resistance, the NANC component is under normal circumstances of little physiological importance. However, it is probable that the structural changes which occur in the obstructed bladder result in significant changes in the physiological response of detrusor muscle (Sibley 1984) and its pharmacological profile (Sjogren et al. 1982) which may indeed be related to the altered behaviour of the obstructed detrusor.

3.2.2 The role of the Sympathetic nervous system.

Langley and Andersson (1895) in their investigation of the innervation of the cat bladder reported that stimulation of the sympathetic neural innervation to the bladder via the hypogastric nerves resulted in a biphasic response, namely, a brief detrusor contraction followed by relaxation, the former being most pronounced in the region of the trigone.

Elliott (1907) reviewed a number of species and concluded that the hypogastric nerves contained both motor and inhibitory fibres, and that there were marked inter-species variations and also striking differences between the sexes within a species. He suggested that the sympathetic nervous system diminished in importance in higher mammals. Subsequently, it has been recognised that much of the confusion relating to the effect of sympathetic innervation in the cat is attributable to differing responses related to the depth of anaesthesia (MacDonald and McCrea 1930).

Later work has clearly confirmed the presence of a typical biphasic response to sympathetic stimulation, comprising a short contraction followed by a marked relaxation of the urinary bladder, in the cat (Ingersoll et al. 1954, Edvardsen 1968a, Norlen 1977) and dog (Ingersoll and Jones 1958), but not the rhesus monkey (Ingersoll and Jones 1962).

Most animal studies have been carried out in dogs and cats, where an abundant sympathetic innervation of the bladder base and proximal urethra has been reported, with a definite but sparse adrenergic innervation of the bladder body (Raezer et al 1973, Sundin and Dahlstrom 1973). It has been suggested by some workers that sympathetic fibres exert a tonic inhibitory influence on the bladder, hence the decrease in end-filling volume and increase in bladder tone produced by sympathectomy (Edvardsen 1968b, Wein et al 1974). Although Klevmark (1977) could not reproduce these findings Nishizawa et al (1985), in a study of the dog bladder, found that hypogastric nerve transection resulted in a small but significant reduction

in bladder end-filling volume and pressure and in initial voiding pressure, with an increase in bladder compliance but with no effect on urodynamic voiding parameters. Nevertheless, in a cat model, increased activity has been demonstrated in the sympathetic neurons to the bladder during filling (Edvardsen 1968a).

Following the functional classification of sympathetic nervous system receptors into α and β adrenoceptor sub-types (Ahlquist 1948), attention has been directed towards determining their distribution and the correlation of this with pharmacological responses. In vitro studies have confirmed that there is a very pronounced regional variation in response to agonist.

In studies conducted in the dog, noradrenaline produced contraction of muscle strips from the bladder trigone and relaxation of tissue from the bladder dome (Rohner et al. 1971). To explain this, it has been suggested that at the trigone there is a functional predominance of α receptors which mediate contractile responses and in the bladder body of β receptors (Edvardsen & Setchlerr 1968) which produce relaxation. Further characterisation studies have confirmed that α receptors predominate at the bladder base and β receptors in the detrusor muscle of the bladder dome (Awad et al. 1974, Wein and Levin 1979).

The pioneering work of Learmonth (1931) confirmed that there was a similar situation in man. He reported that faradic stimulation of sympathetic nerves produced a contraction of the ureteric orifices, increased tonus in the trigone and contraction of the bladder neck, prostatic

musculature and musculature of the seminal vesicles and ejaculatory ducts; but produced no observable effect on the musculature of the bladder walls and dome. Conversely, following division of the sympathetic nerves to the bladder, the ureteric orifices, trigone and bladder neck relaxed, but after three weeks appeared to regain their tone.

Subsequent detailed analyses of adrenoceptor sub-types in the human bladder has resulted in some debate as to their distribution. Some authors maintain that there are no α receptors detectable in the normal human detrusor (Sundin et al. 1977, Nergardh and Boreus 1972). In contrast, Awad et al. (1974) noted that there are both α and β receptors within normal detrusor; a finding corroborated by the functional studies of Todd and Mack (1969) who reported contractile responses to α stimulation and relaxation with β stimulation in the normal human detrusor. Functional characterisation of β adrenoceptors in the human bladder has suggested that they have neither β_1 nor β_2 characteristics (Nergardh et al 1977, Larsen 1979).

Learmonth (1931) reported, in an experiment conducted on himself, that the intravenous injection of noradrenaline produced bladder relaxation. Several investigators have demonstrated a relaxation response attributable to β adrenoceptors in isolated human detrusor (Cowan and Daniel 1983, Todd and Mack 1969, Awad et al. 1974, Nergardh et al. 1977, Larsen 1979). Cowan and Daniel (1983) correlated these findings with the sparse sympathetic innervation to the bladder, and suggested that such responses could result in vivo from the stimulation of adrenoceptors by circulating

catecholamines.

Review of the literature reveals that in clinical trials, β adrenoceptor stimulation and blockade has little effect on the normal detrusor. Beta adrenoceptor agonists (Terbutaline, Isoprenaline) have been reported to produce a small increase in bladder capacity (Norlen et al. 1978). In another study, propranolol decreased rather than increased intravesical pressure in normal man and had no influence on bladder capacity (Jensen 1981).

In isometric muscle strip experiments carried out on the human bladder body, α adrenoceptor agonists appear to be without significant effect (Sundin et al. 1977, Awad et al. 1974). Furthermore, contraction induced by transmural stimulation is little affected by α adrenoceptor blockade with phentolamine (Cowan and Daniel 1983). In normal men, α adrenoceptor stimulation with phenylpropanolamine did not result in an alteration of either intravesical pressure or capacity and α blockade (phentolamine /thymoxamine) had little effect (Jensen 1981).

On the basis of available evidence, the most plausible hypothesis is that bladder filling is influenced by β rather than α adrenoceptors. However, the minor and rather variable effects of α and β agonists and antagonists documented in experimental studies make the importance of such a role uncertain in the normal detrusor.

An important alternative physiological pathway is that provided by neuronal interaction within the autonomic nervous system. Although synaptic contacts between adrenergic and cholinergic neurons at axonal and axon

terminal levels have not been described in man, ultrastructural evidence to support such neuronal interaction is provided by the juxta-position of adrenergic and cholinergic neurons within extra-vesical ganglia. It is of interest that recent work reported by Mattiason and colleagues (1987), documented that nerve mediated release of noradrenaline in normal detrusor muscle strip preparations was decreased by the cholinergic agonist carbachol.

It must therefore be concluded that although the basic mechanism of sympathetic neuromuscular coupling within the human detrusor is well documented, there remains considerable debate as to the precise neurophysiological role of the sympathetic nervous system in the control of the normal bladder and the importance of neural interaction with the parasympathetic and NANC sensorimotor nerves within the autonomic nervous system.

3.2.3 The role of Non-Adrenergic, Non-Cholinergic neurotransmission

In recent years a considerable body of anatomical, biochemical and pharmacological evidence (as outlined above) has demonstrated that NANC neurotransmitters are widely distributed within the body and may play an important role alongside the classical neurotransmitters. Much of the information currently available is rather fragmentary and its derivation from a number of animal species does raise doubts as to the advisability and indeed validity of cross-relating these results. The current knowledge on the neuropharmacological actions of many of these compounds with particular reference to the bladder will now be considered.

Primary afferent neurons contain a number of regulatory peptides (Gibbins et al 1987) which include substance P, calcitonin gene related peptide (CGRP), somatostatin (Som), vasoactive intestinal polypeptide (VIP) and neurokinin A (NKA) (Jancso et al 1977, Sundler et al 1985). The submucosal population of peptide-containing nerves demonstrated in the animal bladder is thought to be associated with sensory innervation. Many of the putative neurotransmitters have also been demonstrated to produce motor effects. In the following section the current knowledge on each putative neurotransmitter as relating to the bladder will be reviewed.

Adenosine triphosphate (ATP)

Scanty evidence is available to substantiate the role of these agents in the human bladder. Husted and co-workers (1983) reported from an in vitro study of muscle from the human bladder dome that ATP produced three types of contractile response, the maximal response being approximately one third of that achieved by acetylcholine. ATP and adenine nucleotides initially potentiated, then subsequently reduced nerve-mediated stimulation and reduced acetylcholine-mediated contraction. Since TTX did not influence the responses in any way they concluded that ATP produced this action by a direct effect on bladder smooth muscle cells.

At present it must be concluded that although the role of purinergic neurotransmission is well established in the animal bladder its potential role in man remains undetermined.

Gamma-aminobutyric acid (GABA)

Gamma-aminobutyric acid (GABA), although well recognised as an inhibitory neu2rotransmitter in the central nervous system, has in recent years been identified in peripheral tissues and has been noted to have inhibitory effects on animal bladder both in vivo and in vitro (Maggi et al 1983, 1985).

5 hydroxytryptamine (5-HT)

5 hydroxytryptamine (5-HT) has been ascribed a neurotransmitter role in the human and animal bladder. Holt et al. (1985), Klarskov and Horby-Peterson (1986) demonstrated that 5-HT evoked a dose-dependent and reversible contractile response in the human detrusor and produced dose dependant relaxation of trigone, bladder neck and urethral smooth muscle. It has been postulated that 5-HT derived from blood platelets may act as a neuromodulator within detrusor muscle (Holt et al. 1985).

Histamine

Histamine is known to produce contraction of smooth muscle in a number of sites within the body. Evidence from animal studies suggests that there are H_1 receptors which mediate histamine-induced contraction of the guinea pig bladder whilst H_2 receptors mediate inhibition of non-cholinergic contraction (Kondo et al. 1985). No evidence for a functional role for this agent in human detrusor has so far been reported.

Vasoactive intestinal polypeptide (VIP)

Vasoactive intestinal polypeptide (VIP), a 28 amino acid compound, was

isolated from the gut (Said and Mutt 1970) and has since been demonstrated in the bladder, prostate and urethra of a number of species (Alm et al. 1977, Alm et al. 1980).

A role for VIP in mediating bladder muscle contractility, possibly by an action on post-ganglionic excitatory neurotransmission, has been suggested. Work with guinea pig isolated bladder strips, where VIP produces small contractions and potentiates nerve-mediated responses (Johns 1979), supports this suggestion. In other species VIP inhibits the motility of isolated vesico-urethral preparations (Maggi and Meli 1986) and in in vitro animal studies VIP produces relaxation of detrusor smooth muscle (Levin and Wein 1981). The distribution of VIP-immunoreactive nerves suggests that they may participate in regulating local blood flow and epithelial function (Alm et al 1980).

VIP may have an important role in the control of human detrusor motor function. Gu et al. (1983a) reported a dramatic reduction of VIP immunoreactivity in the detrusor muscle of unstable human bladder as compared to control. Subsequent in vitro muscle strip studies have demonstrated that the application of VIP produced a significant reduction in muscle strip basal tension and the amplitude and frequency of spontaneous contractions in the normal and hyper-reflexic human detrusor (Kinder and Mundy 1985a, 1985c). Klarskov et al. (1984a) reported that VIP exerted a concentration-dependent relaxation of human detrusor which did not appear to be acting via neuromodulation of neural pathways and was likely to be

producing its effects via a direct action on smooth muscle cells. In support of this, the muscular relaxation occurred at a slower rate than that which had been observed following NANC responses resulting from electrical field stimulation (Andersson et al 1983, Klarskov et al 1983). In contrast, studies of VIP from other anatomical sites suggest that it may act by modulation of neural pathways. For example, in the cat submandibular gland VIP possibly potentiates the action of acetylcholine by increasing the affinity of acetylcholine-binding to muscarinic receptors (Lundberg et al. 1980). No evidence to support such a role in man was found by Sjogren and co-workers (1985) in an in vitro study of human bladder strips where effects on neither acetylcholine-induced responses nor contractions induced by electrical field stimulation were evident.

Few in vivo studies of the action of VIP on the bladder have been reported. Andersson et al. (1987) noted in the anaesthetized cat that stimulation of parasympathetic nerves induced a marked increase in VIP output accompanied by a much smaller relative increase in blood flow; they suggested that this discrepancy could be explained by an additional action on bladder musculature. The intravenous infusion of VIP into male and female volunteers (Klarskov et al. 1984b,1987c) has failed to confirm these expectations, with no demonstrable change in urodynamic parameters of bladder function.

Neuropeptide Y (NPY)

Neuropeptide Y (NPY) is a 36-amino acid peptide, widely distributed in

both the peripheral and central nervous systems. It is the peptide present in the highest concentration in the mammalian brain. NPY may be released in conjunction with noradrenaline upon stimulation of sympathetic nerves in man, with apparent pre- and post-junctional effects on the sympathetic control of blood vessels (Lundberg et al. 1985). Recent work using denervation studies in the rat bladder have revealed that there may be a non-adrenergic population of NPY fibres originating from cell bodies in the pelvic ganglia (Mattiasson et al 1985).

NPY has potent biological actions which include the inhibition of nerve-mediated muscular contraction (mouse vas deferens) and vasoconstriction (Adrian et al 1984). NPY occurs in high concentration in the male genital tract and may play a role in erectile function.

To date no pharmacological studies have been carried out on the human bladder; this is an important oversight, since NPY is the neuropeptide present in greatest concentration in the human detrusor as will be seen in chapter 6.

Calcitonin gene related peptide (CGRP) and substance P (SP)

Calcitonin gene related peptide (CGRP) is a 37-amino acid peptide encoded in the calcitonin gene. Calcitonin gene related peptide (CGRP) often co-exists with substance P within cholinergic neurons in the spinal cord. It has been shown to both stimulate and relax smooth muscle in vitro and via potent effects on blood vessels produces vasodilatation. In the central nervous system it potentiates the action of substance P (Goodman and Iverson 1986).

CGRP and substance P seem to be localised to sensory nerve fibres and CGRP has been reported to be a potent inhibitor of substance P degradation (Le Greves et al 1985). CGRP has been localised within the rat and guinea pig bladder (Gibbins et al 1985, Mulderry 1985), but not so far in the human.

Substance P was first isolated from extracts of brain and intestine by von Euler and Gaddum 1931. It was found to cause contraction of intestinal smooth muscle and to lower blood pressure, actions not influenced by atropine blockade. Substance P was proposed as a sensory transmitter by Lembeck (1953) and certainly fulfulls the principal criteria; it is present in sensory neurons, is released on neural stimulation and exerts appropriate effects on post-synaptic cells in the spinal cord.

CGRP and SP have been shown to co-exist in the same nerves in the rat urinary bladder (Sundler et al. 1985). Capsaicin, a substance derived from Hungarian red peppers is known to selectively affect primary unmyelinated sensory nerve fibres (C-fibres) (Szoleranyi 1977). Neonatal treatment with capsaicin leads to a complete degeneration of C-fibres, whereas treatment of the adult animal leads to a depletion of the neural content of both SP and CGRP and a desensitization to further capsaicin (Maggi 1984, Jancso et al 1985). Cystometric investigation during anaesthesia has shown that rats subjected to neonatal treatment with capsaicin have an increased bladder capacity compared to control rats (Maggi et al. 1986) and resultant urinary retention has also been reported (Sharkey et al. 1983).

A preliminary study of the intravesical instillation of capsaicin into 6 patients has reported that it produces a concentration-related reduction in the first desire to void, bladder capacity and detrusor pressure at the onset of micturition. Of particular interest was the finding that all 5 patients with hypersensitivity disorders of the lower urinary tract reported either disappearance or marked attenuation of their symptoms for a few days after capsaicin installation (Maggi et al. 1989).

Recent observations in the rat bladder have suggested that capsaicin treatment may lead to the development of a selective supersensitivity of muscarinic receptors, albeit with no demonstrable cystometric changes (Malmgren 1989, personal communication). Further data indicate that the administration of SP releases acetylcholine from the rat urinary bladder (Andersson 1989, personal communication).

In recent years it has been recognised that a number of compounds belonging to a group of compounds related to SP - the tachykinins (Erspamer et al. 1981), also occur in the mammalian bladder (Maggi and Meli 1986). Albeit both SP and other neurokinins can produce contraction of the rat (Maggi et al. 1986) or the human urinary bladder (Kalbfleish and Daniel 1987, Dion et al. 1988, Erspamer et al. 1981). It has been suggested that SP is unlikely to function as a NANC motor neurotransmitter because selective SP tachyphylaxis is common and the use of a substance P inhibitor failed to reduce NANC responses to field stimulation (Kalbfleish and Daniel 1987).

The physiological consequences of a sensory neurotransmitter having motor effects remain hypothetical and the contractile effects of SP may represent pharmacological rather than physiological responses. Nevertheless, an attractive hypothesis is that when SP is released in response to afferent stimuli it aids muscle contraction not only by a direct effect but also indirectly via a local action on parasympathetic pathways (a possible explanation for the observation that SP releases acetylcholine in an animal model). This would augment the effects of increased activity within local spinal reflexes, thereby increasing the efficiency of detrusor contraction at the time of voiding.

Met- and Leu- enkephalin (m-Enk and l-Enk)

Methionine-enkephalin (m-Enk) and Leucine-enkephalin (l-Enk) are closely related pentapeptides (Beaumont 1983). The two peptides are localised to synaptic vesicles in nerve terminals within the central nervous system and in peripheral autonomic nerves (La Mont and de Laverolle 1981, Miller 1981). M-Enk and l-Enk have been reported to occur in different nerve populations (Larsson et al. 1979). Enkephalin-immunoreactive nerves have been demonstrated in the lower urinary tract smooth muscle and within ganglia of the cat urinary bladder. It has been suggested that urinary bladder motility is depressed by enkephalin-like substances via a direct action on vesical ganglia (Booth et al. 1981, Simonds et al. 1983) and by intrathecal administration (Hisamitsu et al. 1982). Both m-Enk and l-Enk seem to be implicated in the neural control of micturition (De Groat and

Kawatani 1985), but little evidence is currently available to support a substantive role for these agents in the local control of bladder function (Maggi and Meli 1986).

In an in vitro study of pig lower urinary tract muscle and human detrusor, enkephalins did not influence the basal tension or spontaneous contraction of muscle strips, or of contractions evoked pharmacologically. However, both m-Enk and l-Enk significantly inhibited electrically evoked contractions particularly at low stimulation frequencies, m-Enk being 1.4 times more potent than l-Enk (Klarskov et al. 1987a), and it was concluded that there was a presynaptic inhibition of detrusor muscle contraction. In this study up to 30 % of the control of muscular contraction appeared to be NANC mediated. A major criticism of this work is that TTX was not used to quantify the component due to direct muscle stimulation; hence the results obtained could partly be due to a direct depressant effect of enkephalins on detrusor muscle contractile activity. Furthermore, a long pulse duration was used (1 msec) for electrical stimulation, which could produce significant direct muscle stimulation (Sibley 1984).

3.3 SUMMARY.

It is evident from this brief review that our current knowledge of neuropeptide substances and the amine 5-HT is fragmentary and based on observations taken from a number of species. In view of the ubiquitous distribution of these substances it is to be presumed that they do have a functional role in the nervous system. Nevertheless, surprisingly little

information is available as to the distribution of NANC neurotransmitters in the human bladder or prostate in either health or disease. It was one of the principal aims of the work presented here, to investigate the specific histological localisation of such putative neural substances and these studies are reported in chapters 6, 8 and 11.

CHAPTER 4

PATHOPHYSIOLOGICAL CHANGES IN THE OBSTRUCTED BLADDER

4.1 A review of previous experimental animal studies

The first reported studies concentrated on the effects of acute bladder outlet obstruction produced by urethral ligation in dogs and rabbits (Guyon and Albarron, 1890; Shigematsu, 1928; Creevy, 1934). This produced a non-physiological situation which resulted in marked vesical distension accompanied by acute vesical haemorrhage and mural necrosis. In an attempt to mimic the clinical course produced by prostatic obstruction, Duncan and Goodwin (1949) produced gradual obstruction to the urethra by implanting cellophane bands in dogs, thus stimulating an intense fibrotic response around the proximal urethra. However, this technique was unsatisfactory in that the obstruction was so marked that four out of the seven animals so treated died within six months.

Subsequently, there have been a number of experimental studies on the morphological, contractile and functional effects of partial bladder outlet obstruction. Mayo and Hinman (1976) studied the functional and structural changes within the rabbit bladder at six months following bladder neck obstruction. They noted marked structural changes with collagenous infiltration of muscle and disruption of intracellular junctions. Brent and Stephens (1975), also using a rabbit model, recorded the sequential changes associated with obstruction; with an initial collagenous infiltration followed

by an increased muscle cell mass due to hyperplasia (threefold increase in cell number) and hypertrophy (fivefold increase in cell volume). Uvelius et al. (1984) reported that in the initial stages following experimentally induced bladder outflow obstruction in the rat there was pronounced smooth muscle hypertrophy and hyperplasia with an approximately tenfold increase in the total bladder muscle mass by six weeks following obstruction. These changes occurred with marked rapidity with a significant increase in DNA content being evident after just three days. Levin et al. (1984) confirmed the rapidity of the bladder's response to chronic partial obstruction with a ninefold increase in bladder mass as a consequence of hypertrophy after just one week of obstruction.

These reports demonstrate that the bladder's response to obstruction involves both a collagenous infiltration and muscular hypertrophy and hyperplasia. Variation in results is evident between the different studies which may either be due to methodological differences or inter-species variation. The importance of study design is emphasised by two separate reports on the ultrastructural changes following short term bladder distension. Gosling et al. (1977) reported transient oedema and haemorrhage following a three hour balloon distension of the rabbit bladder with no evidence of significant structural changes on either light or electron-microscopy at up to 18 weeks following the procedure. Sehn (1978), using a similar technique in rats and rabbits, documented no changes following two hours of distension but demonstrated evidence of external injury in over

half of the neuronal axons present within the bladder by two weeks after a 6 hour distension.

Despite extensive work, the mechanisms of functional disruption underlying these documented ultrastructural changes remain obscure. Mayo and Hinman (1976) noted that bladder neck obstruction in the rabbit did not appear to alter bladder contractility to pelvic nerve stimulation. Mattiasson and Uvelius (1982) observed that pelvic nerve stimulation resulted in obstructed rat bladders achieving a similar maximum force per unit cross sectional area as controls but at a considerably greater volume. They concluded that hypertrophic rat detrusor therefore exhibited a decreased ability to generate pressures at smaller bladder volumes as compared to control bladder.

Ghoneim et al. (1986) studied rings of rabbit detrusor after three months of partial bladder outlet obstruction, using muscle strip techniques and concluded that the detrusor response varied with the degree of obstruction. These conclusions were based on the observations that the muscle length at which maximum contractility was exerted increased in moderate obstruction and decreased in severe obstruction, where hyperplasia and collagen infiltration were more evident.

Levin et al. (1984) reported that after one week of bladder outlet obstruction produced by the application of a ligature around the bladder neck, there resulted a 50% reduction in in vitro isometric contractile responses to bethanecol and electrical field stimulation and parallel

reductions in muscarinic receptor density (77%) and intracellular ATP levels (71%). Using an in vitro whole bladder preparation, obstructed bladders had an increased capacity (threefold), but exhibited a 72% reduction in the ability of the bladder to empty its contents as compared to control. Although the contractile and metabolic dysfunction appeared reversible and the functional ability of the isolated bladder returned to 80% of control activity by two weeks following relief of obstruction, the residual functional deficit had not resolved after 4 weeks (Levin et al. 1985). Following on from this work Levin and co-workers (1986) in an acute study using a whole bladder preparation have shown that the obstructed bladder requires an increased intraluminal pressure to empty and does so at a slower rate than controls. In addition, the obstructed bladder is more prone to fatigue after repetitive stimulation. More recent work using an obstructed rat model has suggested that in the hypertrophic muscle the force per area had decreased (Uvelius et al. 1988). Subsequent biochemical study of the muscular proteins actin and myosin in the obstructed rat and human bladder demonstrated an increased actin/myosin ratio which appeared to correlate with this decrease in the maximal active force in hypertrophic bladder (Uvelius et al. 1989).

Detrusor instability is a complex functional disorder, which results from the interaction of a number of interrelated ultrastructural and pharmacological changes under the influence of an intact reflex arc. All of the experimental animal studies so far reported are difficult to relate directly to man, not only because of species differences but also in view of the

limitations of the in vitro experimental techniques which are available. The importance of interspecies variation in pharmacological responses have been demonstrated by a recent comparative study contrasting rabbit, pig and man (Sibley 1984a). In recent years, a great deal of attention has been directed to the development of a satisfactory animal model to reproduce the clinical picture seen in man and thereby allow in vivo physiological studies using urodynamics in combination with the application of ultrastructural and in vitro pharmacological techniques.

The first comprehensive validated model was developed by Sibley and co-workers (1985) and utilised the pig. Previous workers had suggested that the pig lower urinary tract exhibited a number of similarities to man (Melick et al. 1978). It is of note that Jorgenson (1983) had previously produced a situation akin to detrusor instability in 6 out of 7 female pigs at 10 weeks following proximal urethral obstruction. Similarly, Sibley produced a situation mimicking detrusor instability, albeit the cystometric pattern produced cannot be said to be unequivocal detrusor instability as this requires full subject co-operation. The pattern suggestive of detrusor instability occurred in nearly two thirds of the animals obstructed in Sibley's study (9/14), two pigs developed low compliant bladders, one pig a picture suggestive of chronic retention and two pigs remained stable. Following relief of the chronic obstruction in 6 pigs (4 of whom had developed instability, reassessment at 3 months demonstrated that two pigs reverted to stable detrusor function and two remained with abnormal filling

cystometrograms. There was no evidence to suggest that this detrusor instability was related to the severity of the bladder outflow obstruction produced. Pharmacological studies on muscle strips obtained from these animals after 3-5 months of obstruction demonstrated appearances consistent with post-junctional supersensitivity, with exaggerated responses to agonists such as acetylcholine and potassium and a reciprocal reduction in the responses observed to nerve mediated stimulation of the bladder (Sibley 1987).

The dynamic nature of the pathophysiological changes accompanying bladder outlet obstruction must not be forgotten. For instance Ekstrom et al. (1986), from work on the obstructed rat urinary bladder, postulated that supersensitivity initially occurred as a consequence of a dilution of the intramuscular nerve fibres by muscle hypertrophy which was subsequently compensated for by an increase in the field of innervation of individual nerve fibres. Conversely, Sibley postulated that this supersensitivity was not due to an increase in muscle bulk consequent upon hypertrophy or hyperplasia, but represented a true partial denervation of the obstructed detrusor muscle and confirmed this with preliminary non-quantitative ultrastructural studies (1984b).

Further support for this denervation hypothesis follows from in vitro experimental studies in the rat, where an almost direct relationship was noted between the fraction of motor nerves left intact in the bladder and electrically induced contractile effects (Carpenter and Rubin 1967), work

which has been confirmed in vivo in the rat (Elmer 1974), and in vitro in the cat (Mattiasson et al. 1983) and dog bladder (Raz 1983).

Speakman et al. (1987) have extended the work with the pig model up to 18 months. Thirty pigs were studied (8 control and 22 obstructed); 17/22 (77%) of the obstructed pigs developed detrusor instability. This work again suggested a post-junctional supersensitivity. Concomitant objective quantitative studies using light and electron microscopy to count AChE-positive and total nerve profiles respectively, revealed a significant negative correlation between the nerve profile count per mm^2 detrusor muscle and the duration of obstruction. It is of particular note that these workers failed to demonstrate any significant physiological or morphological differences between animals with obstructed and stable bladders and those with obstruction and detrusor instability.

Malmgren and co-workers (1987) have subsequently developed a similar model using the rat as a model. Sprague-Dawley rats had their urethras partially obstructed using ligatures and concurrently had intravesical pressure lines inserted which allowed the measurement of cystometric parameters in conscious animals. After six weeks obstruction, isometric muscle strip experiments demonstrated increased spontaneous activity in the bladder of most obstructed rats (83%), again reminiscent of detrusor instability, with significant increases in bladder capacity (25-fold), bladder compliance, micturition pressure and residual volumes. Further studies using this model (Andersson et al. 1988) have demonstrated a decreased

concentration of substance P and increased concentrations of VIP in obstructed as compared to control bladder. Furthermore, obstructed bladder exhibited a reduced response to neural stimulation, with little corresponding change in response to carbachol. Although VIP could not be demonstrated to produce any pharmacological effect, substance P produced contractions of reduced amplitude in obstructed bladder as compared to control. Two weeks following the relief of six weeks of bladder outlet obstruction, Malmgren et al (1988) documented that strips from these rat bladders exhibited an apparent supersensitivity to carbachol and electrical field stimulation, the latter response being reduced below control and obstructed response levels by scopolamine. The previous reduction in contractile response to substance P was reversed towards control levels. They suggested that instability in this model was not related to cholinergic neurons but rather represented increased myogenic reactivity of smooth muscle cells.

Steers and de Groat (1988) carried out partial urethral ligation in female rats which reproduced the previously described changes in urodynamic variables. They examined changes in the central control of the bladder related to obstruction and noted that experimentally induced bladder distension produced no obvious differences in afferent and efferent nerve activity. In contrast, electrical stimulation of the pelvic nerve afferents evoked two distinct reflexes, a supraspinal and a spinal component. Whilst the supraspinal reflex was unchanged, the spinal reflex was present in a significantly greater percentage of obstructed (100%) than sham operated

controls (35%). They suggested that these changes represented a compensatory spinal reflex mechanism triggered by increased bladder wall tension and work necessary to empty the bladder in the presence of outlet obstruction.

Levin et al. 1984 reported a greater than 50% decrease in the response of the obstructed rat bladder both to bethanecol and nerve mediated stimulation, which is supported by Andersson's observations (1988) and is at variance with the pig model (Sibley 1987). This apparent discrepancy may be explained by the suggestion that there is a reduction in the total force exerted by obstructed versus control bladder if allowance is made for the increased bulk of the bladder musculature (Speakman et al. 1988). Alternatively, it may be related to interspecies differences.

Although these animal studies allow the formulation of plausible hypotheses upon which to base a further investigation of the pathophysiology of secondary detrusor instability, they can only be adequately tested on the human detrusor. Such work is extremely difficult to conduct as evidenced by the paucity of literature available on this subject (vide infra- section 4.2).

4.2 Human studies

Miller (1958) noted smooth muscle hypertrophy as a complication of bladder outlet obstruction. Susset et al. (1978) used biochemical techniques to measure the collagen content of the obstructed bladder and provided evidence of a relative fall in the connective tissue component. Gosling and

Dixon. (1980), using subjective evaluation of histological sections from both control and obstructed bladders, observed connective tissue infiltration of smooth muscle bundles but no apparent change in muscle cell size or number. The combined use of morphological and morphometric techniques were subsequently applied to the study of the obstructed detrusor by the same group (Gilpin et al. 1985); and demonstrated the importance of an objective technique to the identification of significant changes in muscle cell size. In this study 12 of the 14 patients studied had connective tissue infiltration as previously reported (Gosling and Dixon 1980) and furthermore, there was significant detrusor smooth muscle hypertrophy; hyperplasia could not be excluded since specimens incorporating a full thickness of the bladder wall were not available. Similar techniques have subsequently been applied to the objective quantification of AChE-positive neurons using the light microscope in patients with no urological or urodynamic abnormality; and have been further validated by the assessment of total neuronal profile using electron microscopy. These studies have demonstrated a significant reduction in neuronal count in the human detrusor with advancing age (Gilpin et al. 1986). Similar quantitative work in the obstructed detrusor using light and electron microscopy with corrections applied for the increased mean profile area of smooth muscle cells (hypertrophy) and increased amounts of connective tissue (collagen infiltration) has demonstrated a highly significant reduction in nerve profiles per square mm in obstructed bladder versus control ($p < 0.01$); with a 56% reduction in AChE-stained nerves (Gosling et

al. 1986). It must however be remembered that this study looked at a mixed group of male and female patients who had not undergone detailed urodynamic investigation. Nevertheless, these findings parallel those reported from workers on the pig model (Sibley 1987, Speakman et al. 1987) and support the suggestion that post-junctional denervation supersensitivity may be contributing to detrusor instability complicating bladder outlet obstruction.

Whilst this hypothesis provides a plausible explanation for the reported findings, a more detailed correlation with the duration and degree of the changes in detrusor function associated with prostatic obstruction and morphological responses of the detrusor to relief of obstruction needs to be carried out. Although a review of the data so far available from the pig model supports the hypothesis of a post-junctional hypersensitivity it does not satisfactorily explain the predilection of some animals to unstable as contrasted to stable detrusor behaviour in the presence of obstruction. This may be related to the small numbers of animals studied, but additional explanations need to be considered.

Other hypotheses advanced to explain a link between detrusor instability and obstruction remain largely speculative. Chalfin and Bradley (1982) suggested that abnormal sensory stimuli from an altered prostatic urethra could provide sufficient afferent input into a local spinal reflex arc to induce detrusor instability. They injected 6 mls of 1% lignocaine into each lobe of the prostate in 15 patients with bladder outlet obstruction (11 unstable, 4 stable) and documented the effects of this procedure on the cystometrogram.

Local anaesthetic prostate block eliminated instability in 10 out of 11 patients and had no effect on the 4 patients with a normal CMG. On the basis of these findings they concluded that surgical resection of the prostate may correct detrusor instability via a primary action to reduce afferent neural impulses from the prostate.

Other workers have supported the view that abnormal afferent input from the obstructed bladder may be of importance to the genesis of detrusor instability. For instance, it has been suggested that stretch receptors in the bladder wall may have a lower threshold in patients who subsequently develop detrusor instability (Higson et al. 1979). However, this study failed to produce consistent effects to the application of lignocaine. Reuther et al. (1983) reported that the instillation of lignocaine altered bladder sensation and increased the volume at which uninhibited bladder contractions occurred. This has subsequently been investigated by Sethia et al. (1987) who reported that the instillation of an alkaline solution into the obstructed unstable bladder resulted in increased capacity and reduced instability. In contrast, lignocaine produced a variable effect on bladder capacity, increasing it in those with a flow rate > than 10mls per second and decreasing it in those with a flow rate < 10mls per second. In addition, it had little effect on instability and in 3 out of 29 unstable obstructed bladders actually worsened the instability, a finding for which no clear explanation is currently available. Further work is required to clarify these observations before final conclusions can be drawn.

In 1978 Rohner and co-workers reported the results of experiments on the effects of chronic bladder outlet obstruction in dogs. In seven out of twelve obstructed dogs (58%), they documented a change in response to adrenergic stimulation of detrusor muscle in the bladder body and dome from a relaxant β adrenoceptor to a contractile α adrenoceptor response. Rohner observed that the severity of the urethral obstruction was directly related to the muscle strip response, with the greatest contractile effects in dogs with the most severe narrowing of the urethra. They postulated that these pharmacological changes may contribute to the decreased bladder capacity and associated increase in detrusor instability seen in the obstructed bladder. In contrast, Sibley 1987 could not elicit any such alteration in the response of the detrusor muscle to adrenergic stimulation in the obstructed pig model.

A subsequent study of human detrusor muscle obtained from the bladder dome in 47 subjects was reported by Perlberg and Caine (1982). They carried out detailed urodynamic assessment using cystometry in 27 of the patients studied (11 unstable, 16 stable). Of the 11 patients with unstable detrusor behaviour 6 (55%) showed an α adrenoceptor response, 3 (27%) a β response and in 2 (18%) no response; in contrast, in the stable detrusors 15 (94%) exhibited a β response and 1 (6%) an α response. They reported a correlation between the irritative symptoms reported by the patients and a tendency to a contractile α adrenergic response. Reuther and Aagaard (1984) reported a small series of 4 patients; in 3 detrusor instability disappeared

on treatment with the α adrenoceptor antagonist phenoxybenzamine and reappeared when treatment was discontinued. They documented the same findings following surgical relief of obstruction in these patients.

Atropine-resistant non-adrenergic, non-cholinergic neurotransmission may be important in the genesis of detrusor instability. Evidence for this suggestion can be found in studies of detrusor muscle strip preparations from patients with prostatic obstruction reported by Sjogren et al. (1982) and Nergardh & Kinn (1983). Subsequent studies have refuted this suggestion. Sibley (1984b) in a study of 5 obstructed patients reported that an atropine resistant response of 10-20% persisted at 50 Hz, but appeared to be TTX-resistant, suggesting an increased sensitivity of obstructed bladder muscle to direct stimulation. Similarly Kinder and Mundy (1985c) reported almost total inhibition of the response to nerve-mediated stimulation by atropine in normal bladder and subsequently in idiopathic unstable and hyperreflexic detrusor.

The observation that detrusor muscle might be more excitable (Malmgren et al. 1988b, 1989) could be explained by a non-specific increase in muscle excitability in these patients; alternatively there may be a disorder of intrinsic inhibition or neural modulation, which may under normal circumstances be mediated by putative neuropeptide transmitters such as VIP (Gu et al. 1983, Kinder and Mundy, 1985a, 1985c).

Idiopathic instability cannot be distinguished on the basis of filling cystometry from instability occurring in association with bladder outlet

obstruction. Although it is likely that detrusor instability associated with obstruction may be in some way related, it is likely that detrusor instability complicating outflow obstruction represents the final common pathway by which a number of potential pathophysiological mechanisms find their expression. Nevertheless, it is informative to document some of the altered physiological responses reported in the context of idiopathic detrusor instability as they provide an additional perspective.

In complete contrast to the findings reported with the obstructed bladder, Eaton and Bates (1982) reported an increased contractile response by detrusor strips from patients with idiopathic instability to nerve mediated stimulation but with no change in sensitivity to acetylcholine. Kinder and Mundy (1987) documented an increased sensitivity of detrusor muscle in patients with idiopathic instability and hyperreflexia at lower frequencies of stimulation but with no increase in the maximum contractile response produced. The abnormal muscle exhibited an exaggerated response to stimulation with the agonist acetylcholine at threshold concentrations ($< 3 \times 10^{-8}$M), and in addition showed a greater tendency to develop spontaneous contractile activity, higher basal tensions and contractions of greater frequency and amplitude, as compared to normal muscle. These findings suggest a primary myogenic abnormality in unstable muscle. Maximal responses could be largely abolished (92.7 - 97.4%) following pretreatment with atropine, suggesting the principal functional neural component to be parasympathetic, with a limited role for non-adrenergic and non-cholinergic

mechanisms.

4.3 SUMMARY

The current literature supports a number of hypotheses, advanced to explain the development of detrusor instability in association with bladder outlet obstruction. These can best be considered in two categories, myopathic and neuropathic.

Support for a primary myopathic abnormality is provided by the well documented histological changes which occur in the obstructed bladder; the interpretation of the pharmacological studies remains controversial. In vitro animal studies have demonstrated both detrusor underactivity and hyper-excitability. Recent animal models using the rat, lend particular support to the suggestion that increased muscle excitability results from outlet obstruction. This observation is supported by in vitro muscle strip studies in the human.

Neuropathic hypotheses encompass both changes in local spinal reflex pathways and the local bladder innervation, to explain the altered detrusor function associated with detrusor instability. Recent evidence from a rat model suggests that local spinal reflexes are augmented in the presence of bladder obstruction, with exaggerated local spinal reflexes to electrical stimulation of afferent nerves. Histological study suggests that the obstructed bladder undergoes a cholinergic denervation. In vitro pharmacological isometric muscle strip studies have demonstrated post-junctional cholinergic denervation supersensitivity, an increase in NANC-mediated detrusor

contractile activity, reduced contractile responses to substance P and reversed responsiveness of obstructed detrusor to adrenergic stimulation.

As has been emphasised in this review, no comprehensive studies of the histological and physiological changes occurring in the obstructed human bladder have previously been reported. It was the intention of the study to carry out such an investigation with the intention of clarifying this complex and controversial subject.

CHAPTER 5

POSSIBLE PATHOPHYSIOLOGICAL MECHANISMS UNDERLYING DETRUSOR INSTABILITY

5.1 Introduction

Benign prostatic hyperplasia (BPH) is a common problem in the elderly male, occurring with a prevalence of 37-40% in the fifth decade of life rising to 75-84% during the eighth decade (Moore 1935, Franks 1954). It is well recognised that the clinical picture of benign prostatic hyperplasia can remain static for many years (Ball et al. 1981); nevertheless, the risk of a 40 year old patient requiring surgery during his lifetime may be as high as 29% (Glynn et al. 1985).

Secondary detrusor instability complicates prostatic outlet obstruction in up to 80% of patients and prostatectomy results in relief of the bladder overactivity in up to two thirds of patients (Abrams 1985). This altered bladder behaviour is of great clinical importance since symptoms directly attributable to detrusor instability are the commonest cause of patients seeking medical advice and it is of primary importance in causing post-operative urinary incontinence (Fitzpatrick et al. 1979).

Morphological studies in both animals and man have documented significant structural changes with collagenous infiltration of the bladder, muscular hypertrophy and hyperplasia (Uvelius et al.1984, Ekstrom et al. 1986, Gilpin et al. 1985). Objective quantification of acetylcholinesterase

(AChE)-positive nerves has demonstrated a highly significant reduction in nerve count in the obstructed human bladder as contrasted to control (Gosling et al. 1986). The development of suitable animal models which exhibit similar (but not necessarily equivalent) reversible detrusor instability (Sibley 1985, Malmgren et al. 1987) has allowed a search for the possible local underlying mechanisms that may be involved.

The investigation of neurophysiological and pharmacological responses accompanying bladder outlet obstruction has been based on animal models with concomitant pharmacological muscle strip studies (Mattiasson and Uvelius 1982, Levin et al. 1984, Ghoneim 1986). Surprisingly little study of the obstructed human bladder has so far been carried out. A number of hypotheses, which can best be considered as falling into two categories, have been suggested to explain the apparent causal link between bladder outlet obstruction and detrusor instability. Neuropathic hypotheses include the following:- an alteration in bladder adrenoceptor function (Perlburg and Caine 1982, Eaton and Bates 1982), a cholinergic denervation resulting in secondary post-junctional cholinergic supersensitivity (Sibley 1987) and disordered prostatic afferent nerve activity (Chalfin and Bradley 1982, Steers and de Groat 1988). A myopathic hypothesis finds support in the disruption of detrusor muscle seen in ultrastructural studies but is the subject of conflicting reports within the literature (Levin et al.1985, Sibley 1987, Malmgren 1989b).

The intention of this combined histochemical and pharmacological study

was to investigate the changes in cholinergic and adrenergic detrusor innervation and in vitro pharmacological responses occurring in the human bladder following prostatic bladder outlet obstruction. These results were correlated with bladder function assessed both clinically and using comprehensive urodynamic investigation.

5.2 Methods and materials.

5.2.1 STUDY POPULATION

All patients entered this study with full informed consent. The study population comprised patients undergoing surgery for the relief of benign prostatic outflow obstruction in whom there was no evidence of renal failure or co-existing prostatic neoplasia.

The study population was defined using standard videocystometric techniques and fast fill cystometry (50-100ml/min), as described previously (Bates et al. 1970, 1971a, 1971b)- vide infra. Full urodynamic studies were carried out wherever possible on all patients except those in the control group. In view of the current uncertainty over the interpretation of the phenomenon of compliance, all patients with low compliance were excluded from the study leaving patients with stable detrusor function or classical detrusor instability.

Technique for videocystometry.

The technique used is based on that first reported from The Middlesex Hospital (Bates, 1970, 1971a, 1971b). Patients, excluding those with indwelling catheters, were asked to void into a Dantec 5,000 flowmeter to

allow measurement of a free flow rate. They were then requested to lie in the left lateral position on an X-ray screening table whilst a 2mm diameter saline-filled catheter was introduced into the rectum, the end of the tube being protected with a finger stall to prevent faecal blockage. With the patient in the supine position, the external urethral meatus was cleaned with Savlon solution. The urethra was anaesthetized with 1% lignocaine gel containing chlorhexidine. A 10Ch Nelaton filling catheter with a 1mm diameter saline-filled plastic pressure catheter inserted into the sub-terminal site hole was gently inserted into the bladder and the two catheters then disengaged. The bladder was drained of urine and this initial residual volume recorded.

The urodynamic apparatus used was the Ormed 5000 system coupled with an X-ray screening machine (Siemens) (Figure 5.1a). The principles of the technique used are demonstrated on the accompanying schematic diagram (Figure 5.1b).

The two pressure measurement lines were then connected to the transducers incorporated in the urodynamic apparatus. The lines were flushed through with saline, great care being taken to exclude all air bubbles from both the tubing and transducer chambers. Contrast medium (Urografin 150) was then instilled into the bladder at a rate of 100 ml/min under the control of a peristaltic pump. The bladder was filled initially in the supine position and the volume at first sensation of filling was noted. When the subject first experienced discomfort, the radiographic table was tipped

towards the standing position and subsequent bladder filling discontinued when at the maximum tolerated capacity. During bladder filling the patient was asked to consciously suppress bladder contraction. The Nelaton filling catheter was then removed from the bladder and the patient turned to the oblique position relative to the X-ray machine and asked to void into the flowmeter provided.

Throughout the study continuous rectal pressure, total bladder pressure and electronically subtracted detrusor pressure (total bladder pressure minus rectal pressure) measurements were sampled at one Hertz and the results displayed on the VDU and digital display, with hard copies on a five-channel polygraph chart recorder. The adjacent X-ray screening apparatus is linked to a microcomputer which allows the synchronous display of pressure and flow, and also radiographic data relating to bladder morphology, ureteric reflux and the appearances of the bladder outlet and urethra, to be displayed alongside the numerical data on a video display unit. The monitor images were recorded on videotape allowing review and detailed study.

During the early stages of this study a specifically designed computer aided data storage and analysis system was perfected in association with a colleague (Malone-Lee and Chapple 1987). This provided a database facility for patient history, urodynamic data and radiographic data. Analogue information sampled at 1 Hertz and recorded during the urodynamic study was stored automatically to disk. At the end of the study a final report could be issued appended with the investigator's analysis and including

Figure 5.1a

Video-urodynamic laboratory (see Figure5.1b)

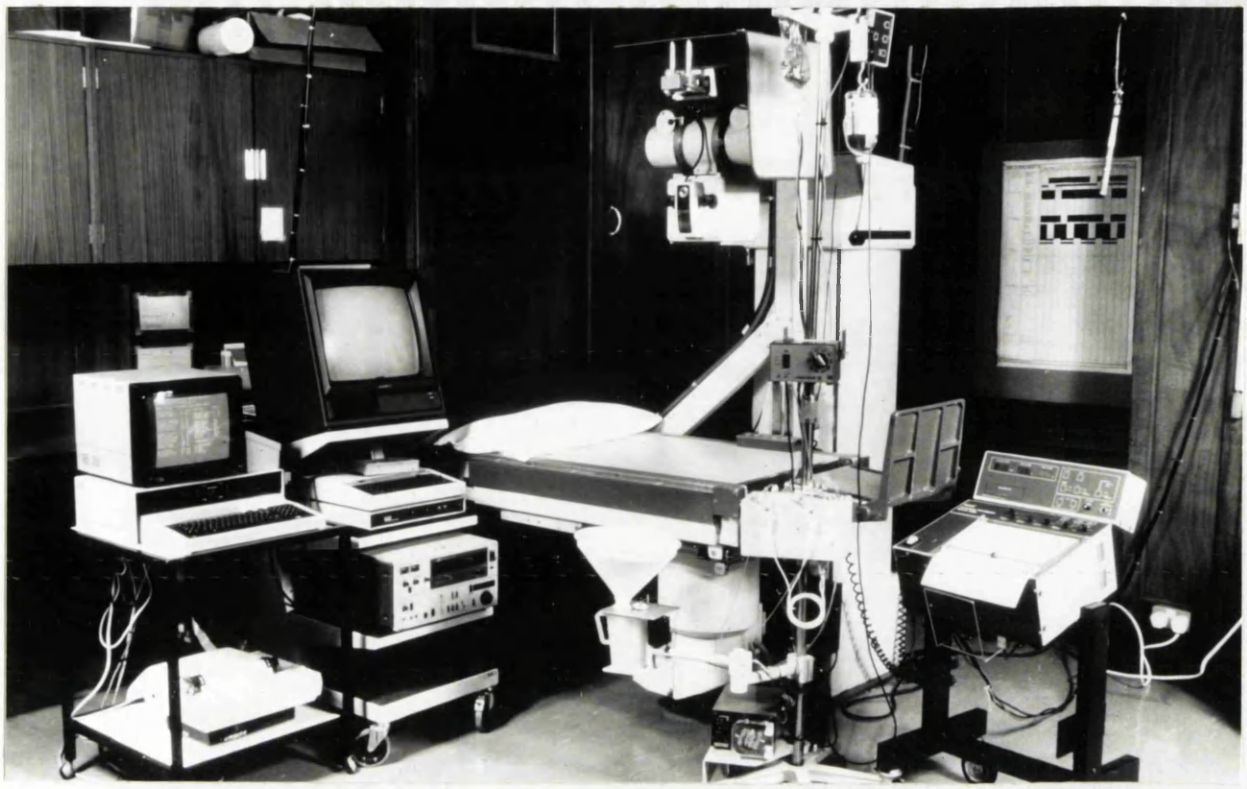
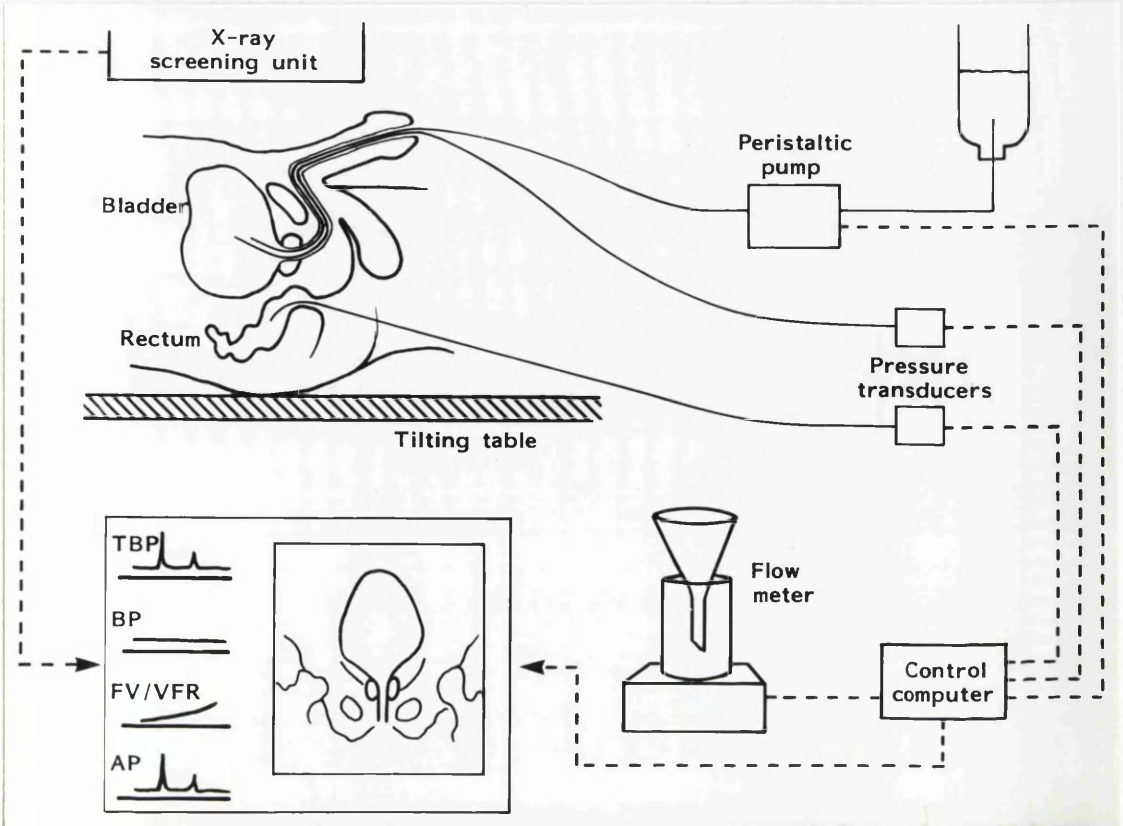


Figure 5.1b.

Diagrammatic representation of the equipment required to perform videocystometry. The bladder is filled at a predetermined rate with a radio-opaque contrast medium with the simultaneous measurement of bladder pressure (TBP) and rectal pressure (AP). The true detrusor pressure (BP) is calculated automatically (TBP-AP). Infused volume (FV) and the voiding flow rate (VFR) are recorded. This information with accompanying radiographic pictures and a sound track is recorded on video tape, allowing for subsequent review and analysis.



appropriate graphic representation of the data.

5.2.2 TISSUE COLLECTION.

Since the validity of the comparisons drawn in this study are dependent upon the control population studied, control bladder tissue used in this study had no clinical, urodynamic or histological evidence (as assessed under blind conditions) of obstruction.

a. Pharmacological studies

Bladder tissue was obtained at the time of open prostatectomy from the lateral wall to the bladder dome, below the peritoneal reflection. Pre-operative subdivision on the basis of urodynamic testing produced the following groups:- stable obstruction (n=3, mean age 60.33 ± 8.76yrs); unstable obstruction (n=6, mean age 70.33 ± 3.60yrs); and acute retention (n=4, mean age 70.25 ± 3.47 yrs).

In addition, control material was obtained from the bladders of patients undergoing other surgical procedures; these subjects had normal flow rates and there was no clinical or histological evidence of bladder outlet obstruction (n=3, mean age 55.00 ± 0.12 yrs).

None of the patients in any of the study groups was on anti-cholinergic or anti-adrenergic medication or received muscarinic receptor antagonists during the course of anaesthetic induction.

b. Histochemical Studies

Bladder tissue was collected from the same patient population specified above and in addition from a number of men undergoing trans-urethral

prostatectomy, using Storz cold punch biopsy forceps. Each biopsy specimen was obtained immediately following surgical removal and fixed to a small piece of cork using the tissue mounting solution "Tissuetek".

i) Some tissue was then snap frozen in theatre in isopentane (2-methylbutane) which had been previously cooled directly in liquid nitrogen to -160°C. The frozen tissue was then labelled and preserved in a store at -70°C. The frozen tissue was transported to laboratories either packed on dry ice or in liquid nitrogen and again stored at -70°C. This tissue was then divided into 2 portions for the following investigations:-

(1) Acetylcholinesterase (AChE)staining.

Tissue was removed from the -70°C store and allowed to equilibrate for 5 minutes at -20°C in a cryostat. Serial sections were cut from each tissue block with a microtome at thicknesses of 10-20μ.

The following groups were studied:- Stable obstruction n=12 (mean age 63.08 ± 2.69yrs); unstable obstruction n=28 (mean age 66.86 ± 1.4yrs); control n=9 (mean age 60.44 ± 5.07yrs); acute retention n=6 (mean age 71.67 ± 2.75yrs); post-prostatectomy n=3 (mean age 72 ±5.51yrs); chronic retention N=4 (mean age 65 ±3.24yrs).

(2) Noradrenaline assay.

Control n=5 (mean age 64.8 ± 8.28 yrs); unstable obstruction n=6 (mean age 68.33 ± 3.13yrs).

ii) A further specimen of the bladder tissue was fixed rapidly in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) pH 7.1-7.4 for

1.5 hrs at room temperature, rinsed in PBS containing 7% sucrose and 0.1% sodium azide, and left overnight at 4°C in the same buffer. The specimen was stained for dopamine β-hydroxylase immunoreactivity. The groups studied were as follows:-

Stable obstruction n=5 (mean age 65.8 ± 2.65yrs); unstable obstruction n=9 (mean age 67.44 ± 2.69yrs); control n=4 (mean age 56 ± 7.78yrs); acute retention n=5 (mean age 69.4 ± 3.49yrs).

The age distribution in each of these groups was compared statistically using the non-parametric Mann-Whitney test. No statistical difference was evident between the various sub-groups ($p>0.05$).

5.2.3 TECHNIQUE FOR PHARMACOLOGICAL STUDIES.

Strips of bladder tissue approximately 1 cm x 1 mm were cut using the dissecting microscope to allow portions with a significant number of muscle fibres running in one direction to be selected. They were then suspended in 10ml organ baths. The tissues were bathed in Krebs solution of the following composition (mmol/l: NaC1 133, KC1 4.7, NaH_2PO_4 1.3, $NaHCO_3$ 16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52 (Bulbring, 1953). The solutions were gassed continuously with 95% oxygen and 5% $C0_2$ and maintained at 37°C. The tissues were placed under an initial resting tension of 0.5g and allowed to equilibrate for 60 minutes. Changes in isometric tension were recorded by means of a force displacement transducer (Grass FTO3C) and displayed on a Grass polygraph (model 7D). Cumulative dose response curves were constructed for acetylcholine (muscarinic receptors) and noradrenaline (α_1 &

α_2 receptors). Characterisation of receptor responses was aided by the use of the antagonists atropine (muscarinic) and prazosin (α_1), to produce selective neuro-effector junction blockade. Electrical field stimulation was carried out using two platinum ring electrodes, suspended 1cm apart. Pulse trains (0.5 msec duration, 60v) were delivered using a Digitimer D4030 and isolated stimulator DS2. The bladder strips were stimulated using pulse trains of 5 seconds over a range of frequencies with a 5-minute interval between each stimulation. The consequences of pre-incubation with atropine ($3x10^{-7}M$) or prazosin ($3x10^{-7}M$) for 30 minutes were investigated. Tetrodotoxin 10 ($10^{-6}M$) was used to abolish all neurally mediated responses to electrical stimulation and thereby distinguish the component due to direct muscle stimulation.

5.2.4 HISTOLOGICAL TECHNIQUES

Tissue was sectioned and adjacent sections stained for routine histology using Masson's trichrome technique (1929) or to demonstrate acetylcholinesterase-positive nerves using the Gomori technique (1952). Other sections were prepared as described above and submitted to specific immuno-histochemical staining for dopamine β-hydroxylase (DBH). Objective quantification was conducted using the protocol outlined below. Additional tissue was submitted to neurochemical assay for noradrenaline.

a) Routine Histology

Masson's (1929) trichrome technique was employed for routine histological purposes. Sections were air-dried on glass slides for 10-15 minutes and then

processed as follows:

1) Harris's Haematoxylin	5 minutes
2) Washed in running tap water	15 minutes
3) 1% Picric Acid (in 70% alcohol)	10 seconds
4) 1% Ponceau de Xylidine (in 0.5% acetic acid)	2.5 minutes
5) 2% Phosphomolybdic Acid	5 minutes
6) 1% Light Green	45 seconds

Sections were washed in tap water after each of the stages 3-6 then dehydrated through graded alcohols, cleared in xylene and mounted on slides.

b) Tissue Acetylcholinesterases

Sections adjacent to those used for routine histology were processed for cholinesterases using the method described by Gomori (1952). Sections were:
1) Fixed in calcium formol (1 volume 35% formaldehyde solution, 6 volumes 2% calcium chloride, 3 volumes distilled water, and neutralised with an excess of calcium carbonate) for 20 minutes at 4 % and washed in tap water.
2) Pre-incubated for 30 minutes at 20°C in one of the following media:

a. 0.075M phosphate buffer at pH7.4.

b. 10^{-5}M solution of the pseudocholinesterase inhibitor TIPA (tetraisopropylpyrophosphoramide - Koch Light Laboratories) in 0.075M phosphate buffer at pH7.4.

c. 10^{-5}M solution of the AChE inhibitor 1,5- bis (4-allyldimethyl-ammoniumphenyl)-pentane-3one-dibromide (Sigma Chemical Company) in

0.075M phosphate buffer at pH7.4.

3) Washed in tap water.

4) Incubated for 3-18 hours at 37°C in Gomori stock solution containing 2mg/ml acetylthiocholine iodine (Boehringer Mannheim) as substrate. Sections pre-incubated in 1,5-bis (-4 allyldimethylammoniumphenyl)-pentane-3one-dibromide were incubated in the above medium containing, in addition, 10^{-5}M concentration of the inhibitor. The Gomori stock solution was prepared as follows:

2.1g copper sulphate	($CuSO_4.l5H_20$)
2.625g glycine	(NH_2CH_2COOH)
7.0g magnesium chloride	($MgCl_2.6H_20$)
12.5g maleic acid	(CH.COOH: CH.COOH)

1200ml saturated sodium sulphate at 40°C

Brought to pH 6.4 with lM sodium hydroxide.

5) Washed in tap water.

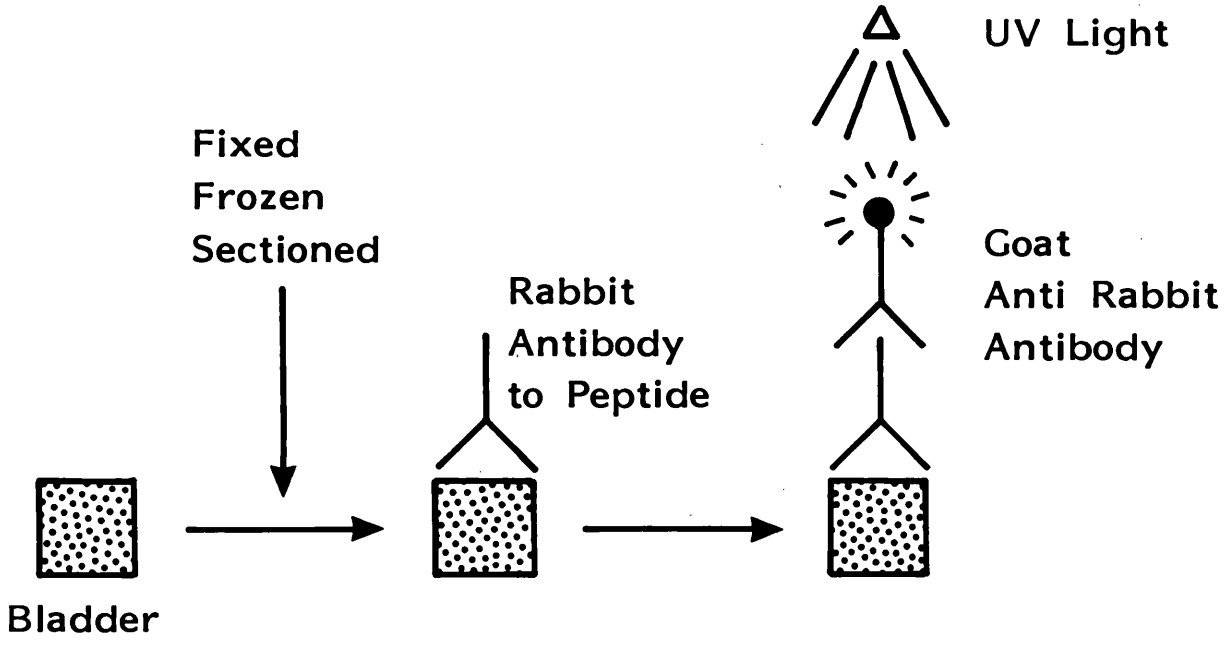
6) Developed in 1% ammonium sulphide solution for one minute and washed in tap water.

7) Counterstained for 30 seconds with Harris's Haematoxylin, and washed in tap water for 5 minutes.

8) Dehydrated through graded alcohols, cleared in tetrachloroethylene and mounted in Canada balsam.

Figure 5.2

Diagrammatic representation of the technique used for indirect immunohistochemical staining.



c) Immunohistochemistry.

The tissue sections (10 µm) were cut on a cryostat and immunofluorescence staining was carried out using the indirect method (Coons et al. 1955, Figure 5.2). The sections were incubated for 18 hours at room temperature with antisera raised in rabbits to dopamine β-hydroxylase (DBH, RIA, Newcastle, U.K.) at a dilution of 1:200, then rinsed in PBS and incubated for 1 hr at room temperature with fluorescein isothiocyanate conjugated sheep anti-rabbit immunoglobulin (Nordic, U.K.) at a dilution of 1:100. After a further PBS wash, the sections were mounted in PBS/glycerol (1:1) and examined using a Zeiss fluorescence microscope fitted with epi-illumination. Control sections were incubated with either normal rabbit serum, or antiserum which had been pre-absorbed with DBH.

d) Neurochemistry

All tissue specimens were frozen and stored in liquid nitrogen until assayed. Immediately prior to assay, samples of bladder were carefully examined and dissected free of extraneous connective tissue. The tissue specimens were then weighed.

Analysis of noradrenaline levels was carried out using high performance liquid chromatography with electrochemical detection. The extraction procedure, slightly modified by the addition of 0.1 mM EDTA in the solution used for washing the alumina, was essentially that of Keller et al.(1976). Chromatography was carried out at a flow rate of 2.0 ml/min. using a mobile phase consisting of 0.1 M sodium dihydrogen phosphate, 0.1 mM EDTA,

5mM heptane sulphonate (pH 5.0) containing 10 per cent (v/v) methanol on a micro-Bondapak C-18 reverse phase column. Detection and quantification were accomplished with a glass carbon electrode set at a potential of + 0.72 V. Noradrenaline levels were corrected for recovery using dihydroxy-benzylamine as an internal standard (Moyer and Jiang, 1978).

e) Quantitative Methods for Light Microscopy

All sections were coded prior to processing and staining and were examined by the observer in a blind fashion.

a. Amount of muscle/mm^2 tissue.

The amount of muscle contained within a given area of detrusor tissue from each biopsy was determined using a point-counting technique (Williams 1977, Figure 5.3). Multiple non-overlapping fields (minimum 5) were assessed. An eyepiece graticule with a grid divided into 1mm squares was used; at each point of intersection the nature of the underlying tissue was recorded. The mean value for the amount of muscle per unit area was expressed as a percentage for each biopsy.

b. Assessment of muscle cell mean profile area (MPA).

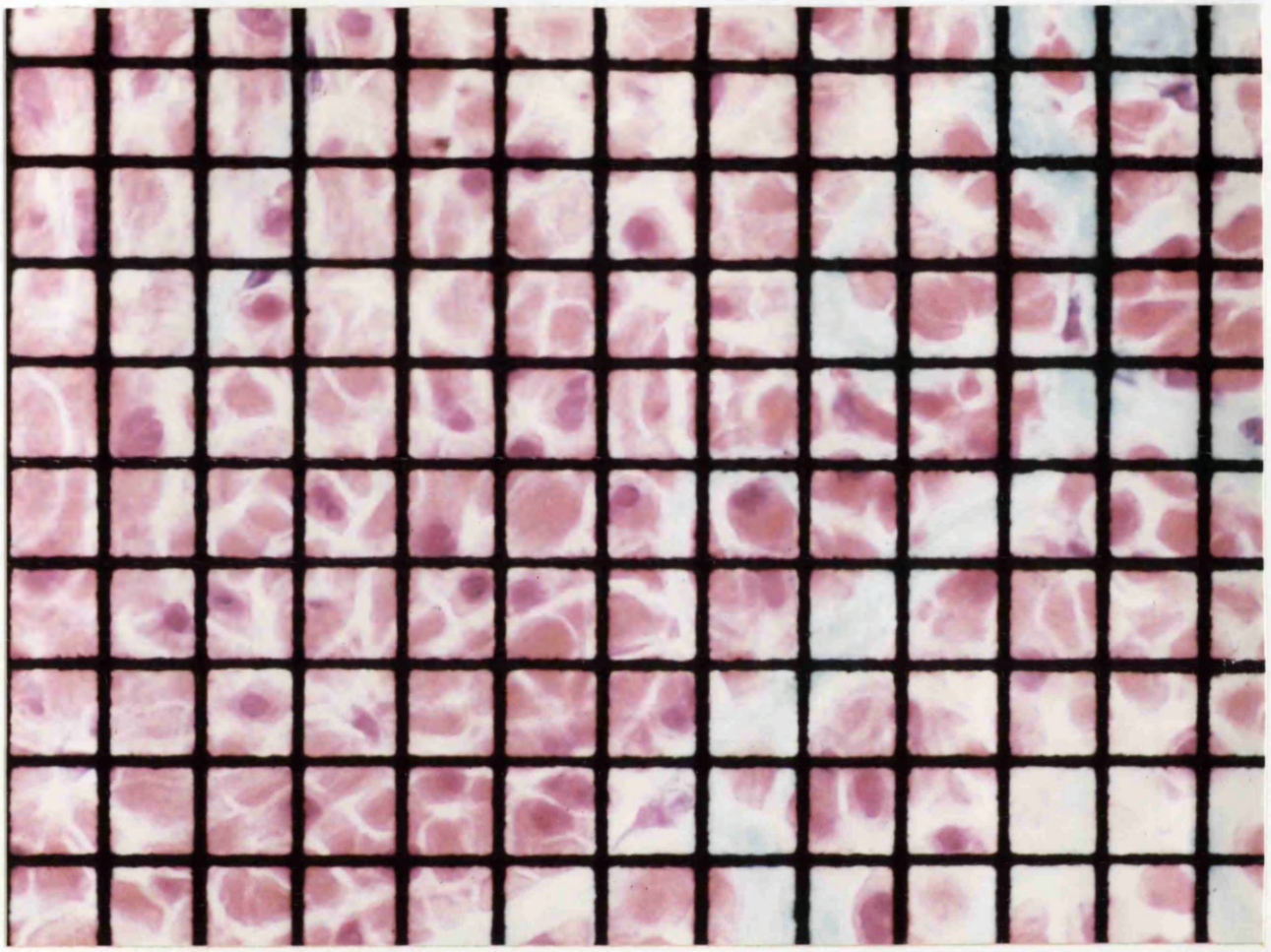
Photomicrographs of transversely sectioned smooth muscle cells were prepared and the total area of smooth muscle determined using a point-counting technique. This was then divided by the actual number of cells present to determine (MPA) for each patient.

c. Amount of nerve/mm^2 tissue.

Sections stained to demonstrate AChE and DBH-like immunoreactivity

Figure 5.3.

Section stained using the Masson technique, muscle shown in pink, connective tissue in blue with superimposed counting graticule (magnification x 100, graticule not to scale).



were examined using the same magnification and the same eyepiece graticule as above (Figure 5.4). Counts were made of the number of points (grid intersections) which overlaid immunoreactive nerves in multiple non-overlapping fields (10, minimum 5) selected so as to cover the entire area of muscle tissue contained in sections from each biopsy. This was extremely important since heterogeneity of staining was evident between different areas within the bladder; this applied to both control (Figures 5.5a&b) and obstructed bladder (Figures 5.6a&b).Different areas were examined, far enough apart to preclude the possibility of counting any point twice. A minimum of 500 and a maximum of 2000 points were counted in every case, and a mean value for the density of innervation per square mm was calculated for each biopsy, and then for each patient. Although an age related loss of bladder muscle nerves has been previously reported, this was not apparent in the control material studied here.

5.2.5 STATISTICAL ANALYSES

All results are expressed as mean ± S.E.M. Statistical analyses were performed to contrast patients with controls and compare results obtained in each sub-group where appropriate. Although in most groups the data approximated to a normal distribution, in view of the small sample sizes non-parametric statistical analysis was performed using the Mann Whitney U test to determine statistical significance. Data analyses were carried out using the Minitab 5.1.1 software package on a Tandon IBM-compatible computer. A level of probability of 0.05 or less was considered significant.

Figure 5.4.

Section stained using the Gomori technique for acetyl-cholinesterase, positive staining nerves shown in dark brown, with overlying graticule for quantification (magnification x100, graticule not to scale).

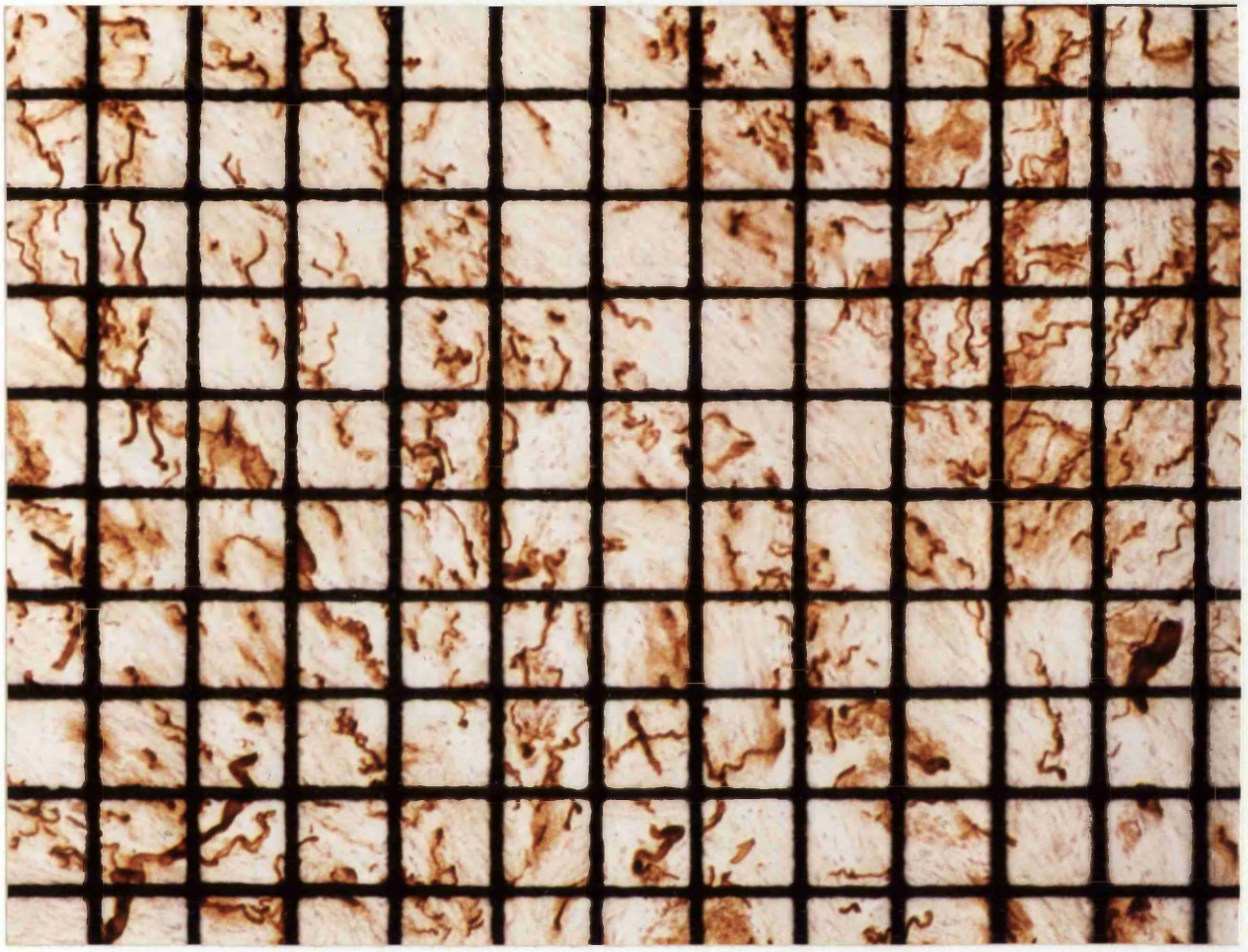
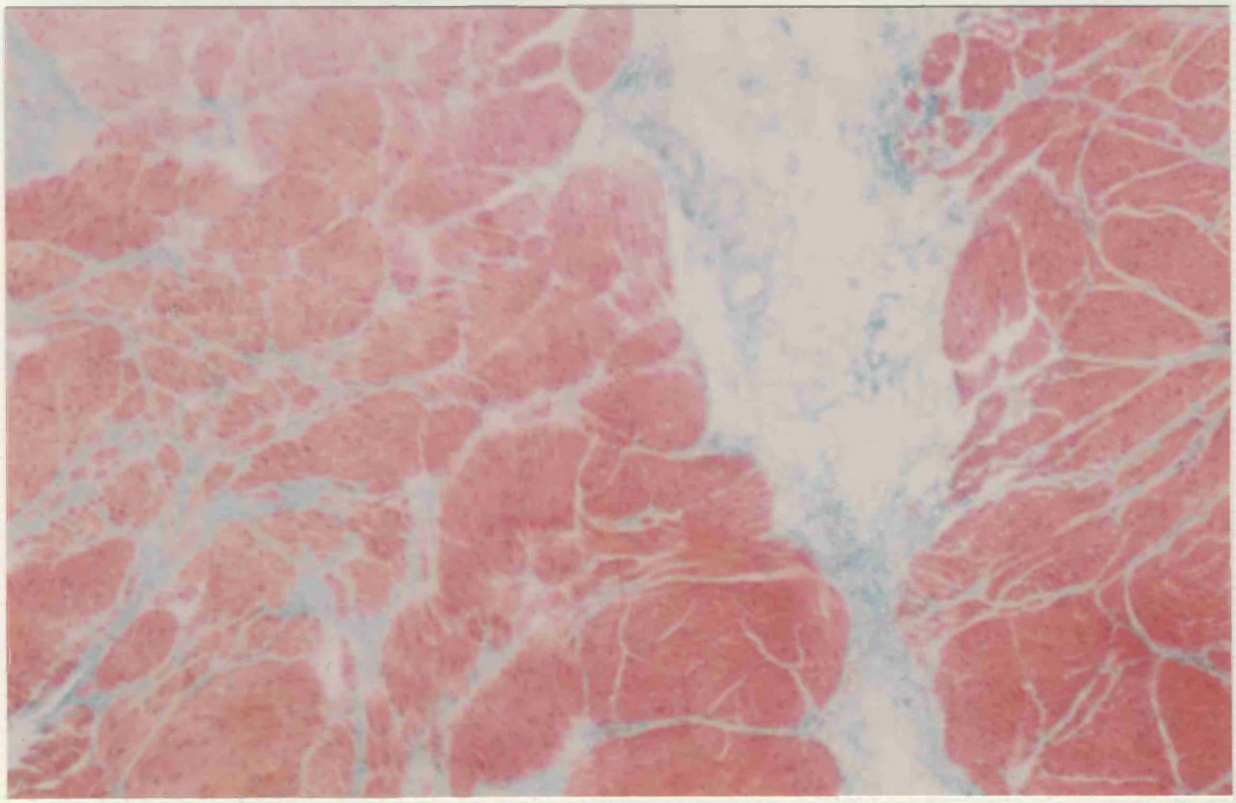


Figure 5.5.

Consecutive sections stained with Mallory Trichrome a) and using the Gomori technique b) demonstrating the variability of AChE staining within control bladder (magnification x90).

a)



b)

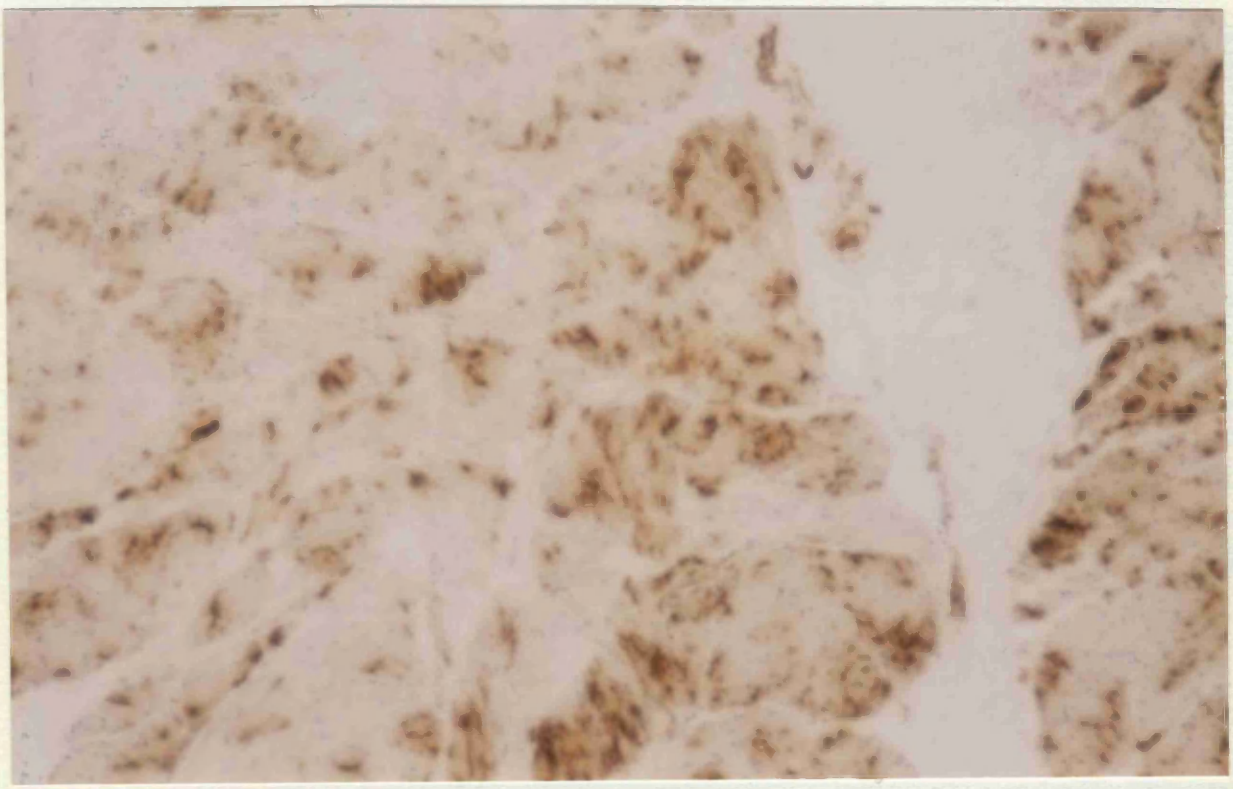
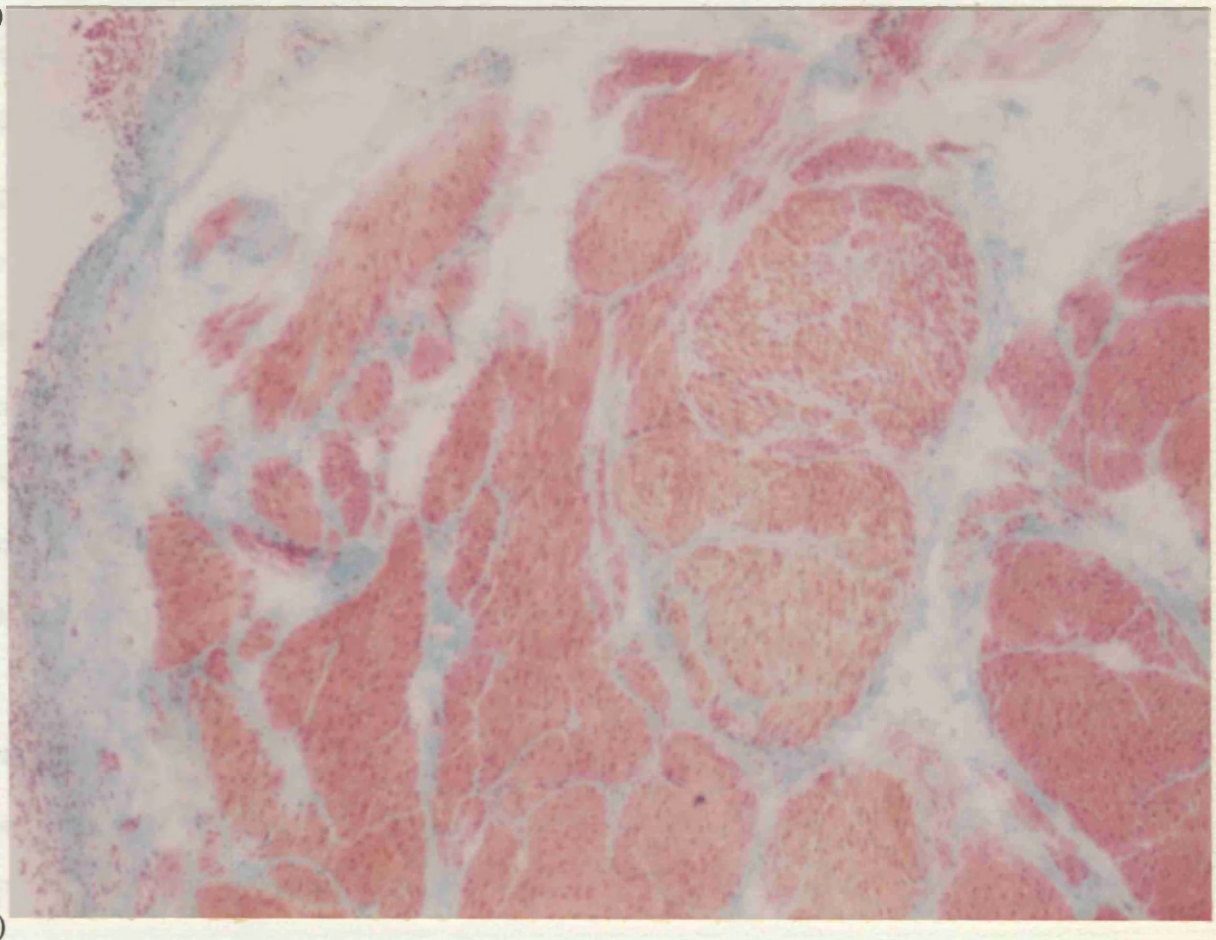


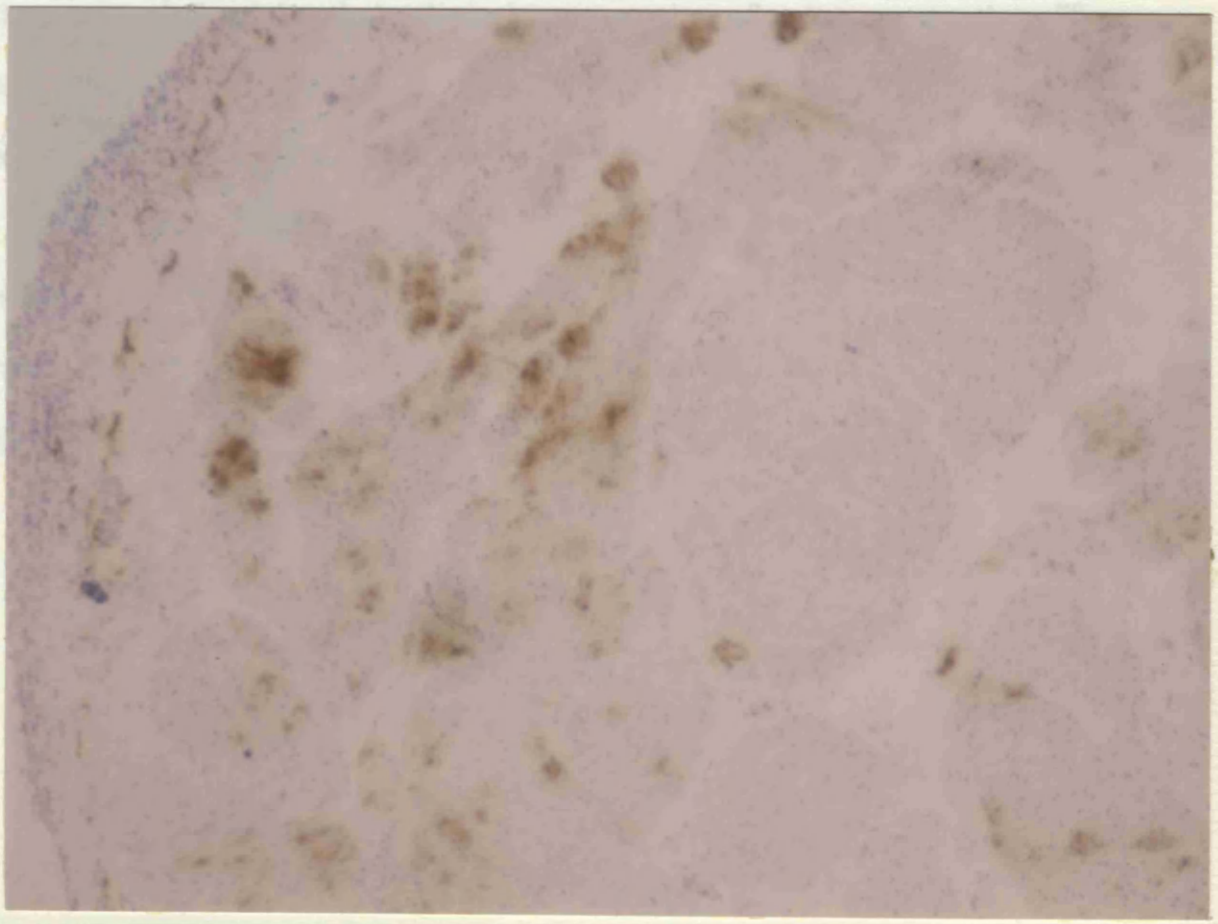
Figure 5.6.

Consecutive sections stained with Mallory Trichrome a) and using the Gomori technique b) demonstrating the sparsity of staining for AChE in obstructed bladder as compared to control(Fig5.5) and the variability of staining (magnification x90).

a)



b)



5.3 RESULTS

5.3.1 Cystometric results (see Table 5.1)

In the control group only free flow rate results were available, the maximum flow rate being 26.75 ± 1.38 ml/sec (voided volume 337.5 ml ± 31.5 ml). The unstable obstructed category (n = 30) represented the largest group studied. The mean duration of obstructive symptoms was 4.18 ± 0.56 y, maximum filling pressure 61.69 ± 6.65 cmH_2O, mean voiding pressure 88.57 ± 6.69 cmH_2O, with a maximum flow rate of 9.76 ± 0.98 ml/sec. In comparison, in the stable obstructed group, the mean duration of symptoms was 3.46 ± 0.93 years, with similar results for all except filling pressure. A surprising finding was that the volume at first sensation for filling and bladder capacity was very similar in both groups.

An interesting observation relating to both the acute and chronic retention groups of patients was that they almost universally exhibited low compliance with markedly reduced bladder volumes during filling cystometry. These patients were not excluded according to the criteria used above as they were defined by their clinical presentation of urinary retention. The most likely explanation is that this abnormal pressure rise was a response of the bladder to being defunctioned by catheterisation.

The three patients in the post-prostatectomy group were being investigated for persistent frequency following prostatectomy. The free flow rates measured were 18, 20 & 15 ml/sec respectively; in 2 of the 3 patients cystometry was carried out revealing a maximum filling pressure of 40 and

24 cmH_2O thus confirming a diagnosis of detrusor instability. In the 2 patients who underwent cystometry the maximum voiding pressures were 42 and 55 cmH_2O. Thus, there was no evidence to suggest that any of these 3 patients were significantly obstructed.

TABLE 5.1
CYSTOMETRIC RESULTS (MEAN VALUE ± S.E.M.)
(mean values only if results available > n=3)

study group	dur. of sympt y	free flow rate ml / sec	init. void. vol. ml	init. residv ol. ml	max. fill. press cm H_2O	vol 1st sens. ml	capac ml	max. void. press cm H_2O	max. flow rate ml / sec	post. mict. resid ml
contr n=4	----	26.75 ± 1.38.	337 ± 31.5.	26 ±10.3.	----	----	----	----	----	----
unst. obs. n=30	4.18 ± 0.56	8.36 ± 0.79	180 ±17.6	59 ±15.4	61.69 ± 6.65	183 ± 15.1	403 ± 28	88.57 ± 6.69	9.76 ± 0.98	99 ±0.4
stab. obs. n=12	3.46 ± 0.93	9.58 ± 1.32	90 ± 30.4	28 ± 13	13.17 ± 0.97	171 ± 23	395 ± 28.3	80.83 ±3.9	12.33 ± 1.82	13 ±5.38
acute ret. n=10	----	----	----	900 ± 139	83.3 ± 10.6	----	201 ±51.5	----	----	----
chr. ret. n=3	0.25 ± 0.25	----	----	1400 ± 227	48.69	----	657	----	----	----
post prost n=3	2.5 ± 0.76	17.67	210	13	32	153	405	48.5	18.5	0

5.3.2 Morphological Studies

AChE-positive nerves/mm^2

The amount of smooth muscle/mm^2 of tissue in the biopsies from obstructed patients was similar in all of the groups. The range of values seen was 53 to 84% (expressed as the percentage of the total tissue bulk occupied by muscle). The following mean results were obtained in each group; unstable obstruction 73.18 ± 1.22%, stable obstruction 72.58 ± 1.89%, acute retention 73.33 ± 2.75%, chronic retention 63.75 ± 6.63%. The corresponding results for chronic retention were significantly lower than those for the unstable obstructed group ($p \leq 0.02$) but not the stable obstruction or acute retention groups. The amount of smooth muscle/mm^2 in biopsies from control patients varied from 75 to 86% with a mean value of 79.56 ± 1.14%. In the small group of patients who were studied post-prostatectomy, with no urodynamic evidence of obstruction, the muscle content was raised as contrasted to other groups ($p<0.05$) (86 ± 3.21%).

The smooth muscle cell mean profile area (MPA) was measured in order to allow correction of results for cell size, using the light microscope technique validated by previous study (Gosling et al. 1986). It was not possible to carry out this measurement in all specimens and therefore random samples from each group were measured. After measurement of the number of nerves/mm^2 tissue the figure calculated for % smooth muscle in each biopsy was used to calculate number of nerves/mm^2 muscle, after correction for differences in MPA (see tables 5.2 to 5.7).

TABLE 5.2

UNSTABLE OBSTRUCTED PATIENTS

PATIENT NO.	AGE(YRS)	% MUSCLE/ mm^2 TISSUE	NERVES/ mm^2 MUSCLE*
1	72	71	110
2	66	74	50
3	54	72	52
4	79	75	7
5	67	55	159
6	70	83	85
7	74	78	131
8	70	65	107
9	60	68	40
10	74	78	103
11	63	73	76
12	61	81	93
13	70	74	21
14	55	68	198
15	56	81	220
16	75	72	137
17	70	71	133
18	78	69	99
19	63	75	62
20	76	73	34
21	53	60	30
22	70	77	77
23	67	78	125
24	65	83	106
25	75	75	105
26	65	68	110
27	66	73	185
28	58	79	104
MEAN ±S.E.M.	66.86 ± 1.4	73.18 ± 1.22	98.54 ± 9.92

* These values are corrected to allow for increases in muscle cell size.

TABLE 5.3.
STABLE OBSTRUCTED PATIENTS

PATIENT NO.	AGE (YRS)	% MUSCLE/ mm^2 TISSUE	NERVE/ mm^2 MUSCLE*
1	68	84	126
2	62	78	34
3	57	74	40
4	80	77	211
5	62	68	73
6	58	75	96
7	66	65	141
8	55	79	183
9	63	74	59
10	74	63	33
11	68	65	66
12	44	69	93
MEAN ± S.E.M.	63.08 ± 2.69	72.58 ± 1.89	96.2 ± 16.8

* These values are corrected to allow for increases in muscle cell size.

TABLE 5.4.
ACUTE RETENTION BLADDER.

PATIENT NO.	AGE (YRS)	% MUSCLE/ mm^2 TISSUE	NERVES/ mm^2 MUSCLE*
1	80	80	102
2	64	74	60
3	67	68	148
4	73	72	207
5	79	60	36
6	67	68	111
MEAN ± S.E.M.	71.67 ± 2.75	70.33 ± 2.75	110.7 ± 25.1

* These values are corrected to allow for increases in muscle cell size.

TABLE 5.5.
CONTROL BLADDER

PATIENT NO.	AGE (YRS)	% MUSCLE/ mm^2 TISSUE	NERVE/ mm^2 MUSCLE*
1	46	82	157
2	73	80	156
3	60	80	289
4	63	81	166
5	29	78	246
6	72	75	407
7	63	75	271
8	59	79	245
9	79	86	129
MEAN ± S.E.M.	60.44 ± 5.07	79.56 ± 1.14	229.6 ± 29.4

* These results are corrected to allow for increases in muscle cell size.

TABLE 5.6
CHRONIC RETENTION BLADDER

PATIENT NO.	AGE (YRS)	% MUSCLE/ mm^2 TISSUE	NERVES/ mm^2 MUSCLE*
1	71	83	11
2	56	53	35
3	65	58	53
4	68	61	0
MEAN ± S.E.M.	65 ± 3.24	63.75 ± 6.63	24.7 ± 11.9

* These values are corrected to allow for increases in muscle cell size.

TABLE 5.7
POST-PROSTATECTOMY BLADDER.

PATIENT NO.	AGE(YRS)	%MUSCLE/ mm^2 TISSUE	NERVES/ mm^2 MUSCLE*
1	83	81	114
2	66	85	159
3	67	92	200
MEAN±	72±	86±	157.7±
S.E.M.	5.51	3.21	24.8

* These values are corrected to allow for increases in muscle cell size.

The results of the quantification of AChE-positive nerves in all of the study groups are summarised in Figure 5.7 (showing the results of non-parametric statistical comparison to control). The most striking observation is the marked reduction in AChE-positive nerves seen in all obstructed groups as contrasted to control ($P \leq 0.01$). Overall, there was a 56% reduction in the nerve count in obstructed bladder, but this was often higher in individual groups, for example, in patients with acute retention where the corresponding reduction was 89%. The most marked reduction in AChE positive nerves was evident in the chronic retention group, this was statistically significant as compared to all of the other groups stable obstructed ($p \leq 0.03$), unstable obstruction ($p \leq 0.01$), acute retention ($p \leq 0.03$), control ($p \leq 0.007$)). The differences in innervation between the stable obstructed, unstable obstructed and acute retention sub-groups were not significant. Innervation of the post-prostatectomy group was significantly higher than in the unstable obstructed patients ($p \leq 0.05$). There was no correlation between patients' ages and numbers of AChE containing nerves/mm^2 muscle (see Figure 5.8). Comparing the results of patient's age and corresponding bladder innervation for the whole population there was no significant correlation ($r = -0.143$). A similar finding was evident within each of the sub-populations.

Figure 5.7.

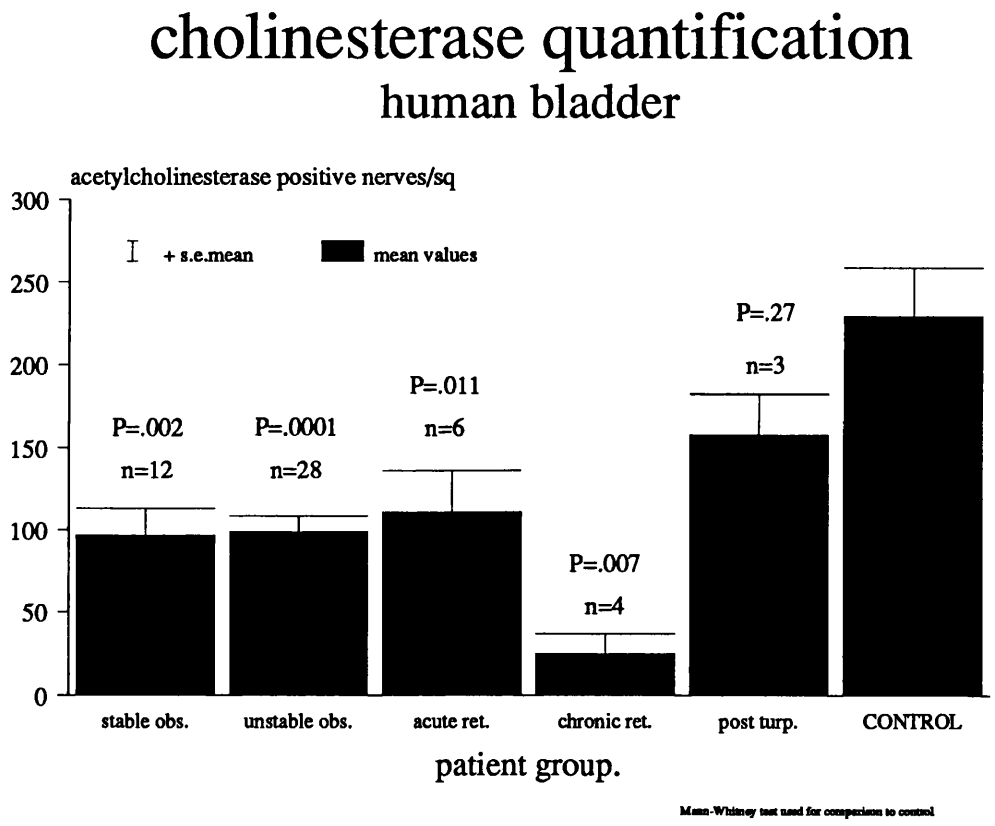
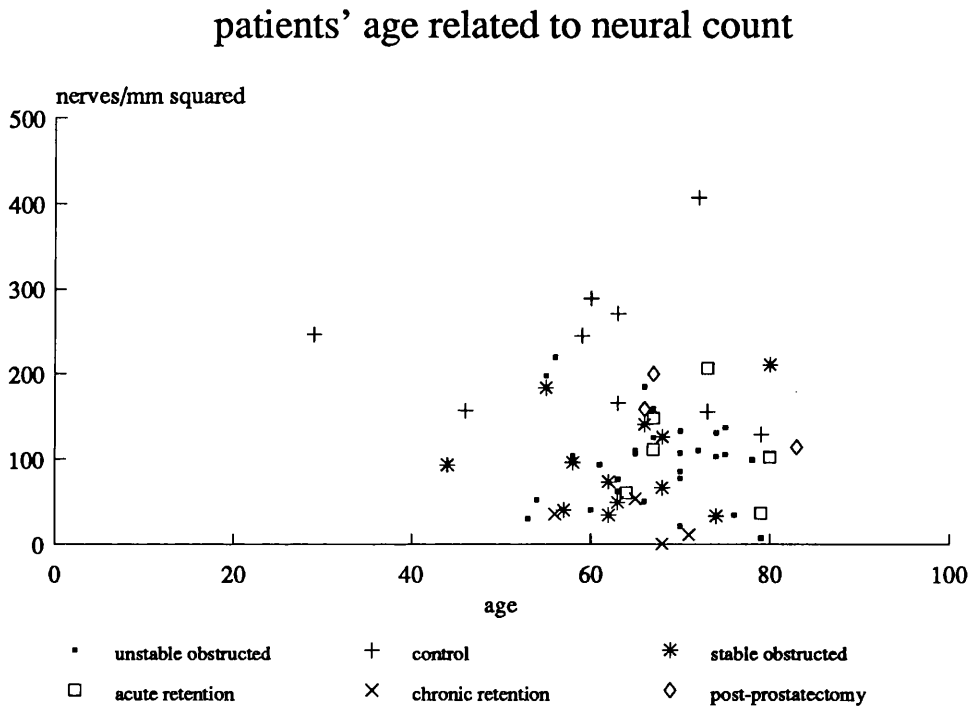

cholinesterase quantification
human bladder
acetylcholinesterase positive nerves/sq
300
250
200
150
100
50
0
+ s.e.mean
mean values
P=.002
n=12
P=.0001
n=28
P=.011
n=6
P=.007
n=4
P=.27
n=3
stable obs.
unstable obs.
acute ret.
chronic ret.
post turp.
CONTROL
patient group.
Mann-Whitney test used for comparison to control

Figure 5.8.



acetylcholinesterase-positive nerves

Dopamine β Hydroxylase Quantification

DBH immunoreactive nerves were sparsely distributed within the bladder wall, running along muscle fibres and also in the mucosa. The results of objective quantification for each group were not statistically significantly different ($p>0.05$):- (expressed as mean ± S.E.M.) control n=4, 4.89 ± 3.67 nerves/mm^2 muscle, unstable obstructed n=9, 4.44 ± 3.93 nerves/mm^2 muscle, stable obstructed n=5, 7.95 ± 7.95 nerves/mm^2 muscle, acute retention n=4, 1.32 ± 1.32 nerves/mm^2 muscle. Although, subjective quantification suggested a reduction in the obstructed groups as contrasted to control (Figure 5.9).

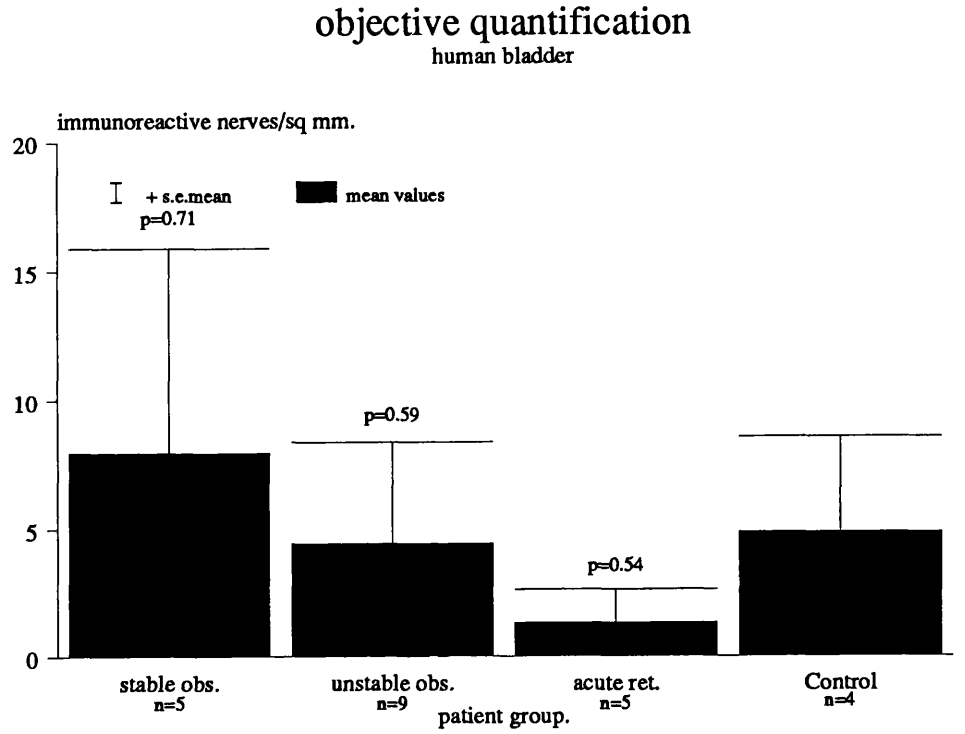
Biochemical Assay Noradrenaline

A small number of patients in the control and unstable obstructed groups had tissue assayed for noradrenaline (Figure 5.10), the mean values obtained were control n=5, 0.07 ± 0.013, µg/g wet tissue and unstable obstruction n=6, 0.16 ± 0.03 µg/g tissue, and this difference was statistically significant ($p<0.05$).

5.3.3 Pharmacological Studies

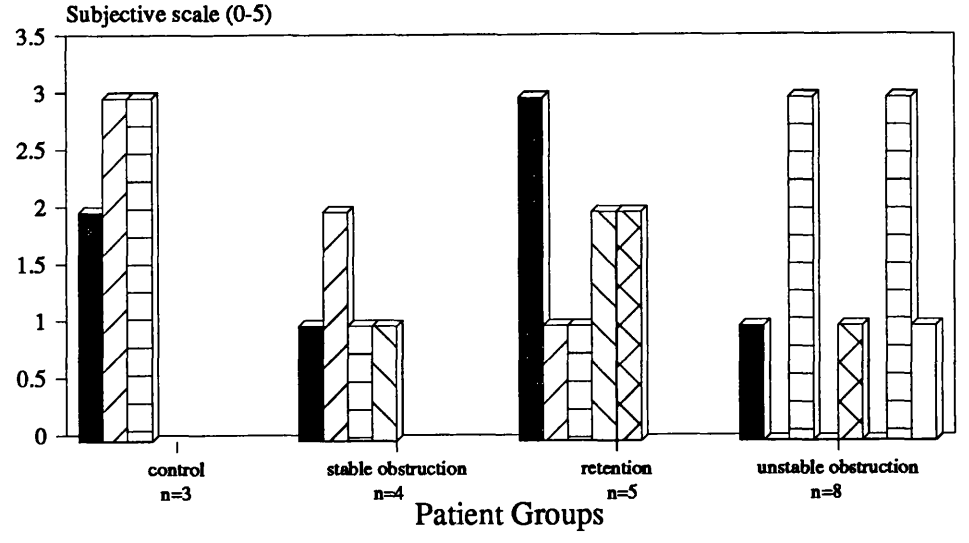
Muscle strip preparations from obstructed detrusor 14/18 (78%), exhibited an increased tendency to spontaneous activity, as contrasted to control 3/7 (43%). The significance of this finding is obscure, since considerable variability in the incidence of spontaneous contractions was often evident in different preparations from the same patient (Figure 5.11). Dose response curves for acetylcholine and noradrenaline were standardised by expressing them as a mean % of the response to maximal stimulation achieved by the

Figure 5.9.



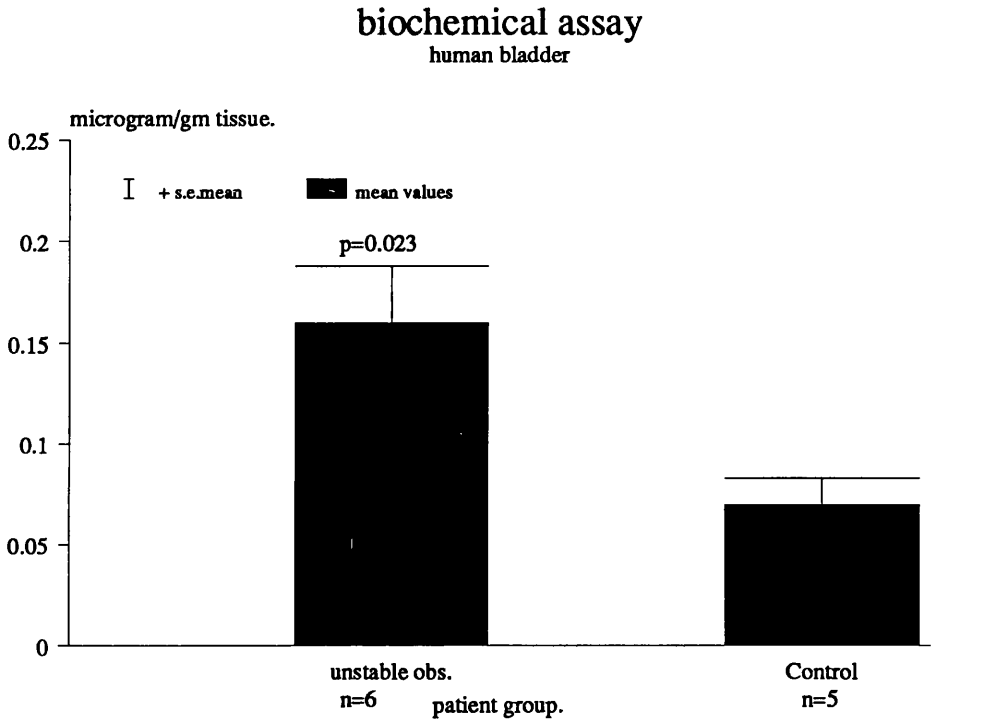
DBH - immunoreactive nerves

Mann-Whitney test used for comparison to control



DBH - IMMUNOREACTIVE NERVES

Figure 5.10



noradrenaline

Mann Whitney test used to compare statistical significance

Variation in the spontaneous contractile response of bladder muscle preparations (a-d).

Table 5.8

STUDY GROUP	PATIENTS	MEAN	± Std. ERROR
Control	3	1.758	0.369
Acute Retention.	4	0.632*	0.104
Stable Obstruction.	3	1.029	0.360
Unstable Obstruction.	6	0.720+	0.253

Maximum response to K^+ (mean ± S.E.M.).

Mann Whitney test
* $p \leq 0.05$

application of potassium (124mM). The mean maximal responses obtained for each group are listed in Table 5.8.

The magnitude of the mean maximal response was much reduced in the obstructed groups as contrasted to control; as can be seen by expressing the results as a % of the control values, unstable obstruction (41%), stable obstruction (59%), acute retention (36%). These differences were statistically significant only in the acute retention group ($p \leq 0.05$) as compared to control.

All viable bladder strips contracted in response to neural stimulation (Figure 5.12) and the application of acetylcholine (Figure 5.13). The application of exogenous noradrenaline to control bladder either produced no detectable response or a relaxation of the preparation (Figure 5.14). In the stable obstructed bladder 4/9 strips in 2/3 patients relaxed. The mean (±S.E.M.) maximum response was -10.22 ± 6.63% at 10^{-5}M and this level of relaxation was sustained throughout the dose range up to 10^{-4}M. In contrast, muscle strips from patients with acute retention of urine or unstable obstructed bladders exhibited a contractile response to noradrenaline, which was seen in 6/11 strips from 3/4 patients with unstable obstruction who were tested and 4/6 (67%) strips from 3/4 patients with acute retention of urine. This contractile response (see Figure 5.15) occurred in both groups of patients at all concentrations of the dose response curve (5×10^{-7}M - 10^{-4}M), the increase in contractile response being most pronounced in those with acute retention of urine. The contractile response to noradrenaline was reduced by up to 80% following incubation with prazosin 10^{-7} M (n= 4) (see

fig 5.16). The specific α-2 agonist UK-14304 produced no contractile effect in all of the preparations from obstructed groups which were studied (n=5). Prazosin did not significantly reduce the effects of neural stimulation in either control tissue (n=2) or obstructed tissue (n=3); there was therefore no evidence that a significant proportion of the response to electrical stimulation was mediated via α_1 adrenoceptors (Figure 5.16). The responses of both obstructed and non-obstructed bladder (n=7, n=3) to nerve-mediated stimulation and acetylcholine were antagonised by atropine in a dose-dependent fashion (Figures 5.17 & 5.18) suggesting that the majority of the contraction was mediated via the release of the neurotransmitter acetylcholine. A reduction in the contractile response which was of similar magnitude, occurred after the administration of tetrodotoxin (TTX) 10^{-6} M (n=5), suggesting that only a small proportion of the bladders contractile response of the bladder could be attributed to non-adrenergic, non-cholinergic neurotransmission (Figure 5.17).

Figure 3.12:

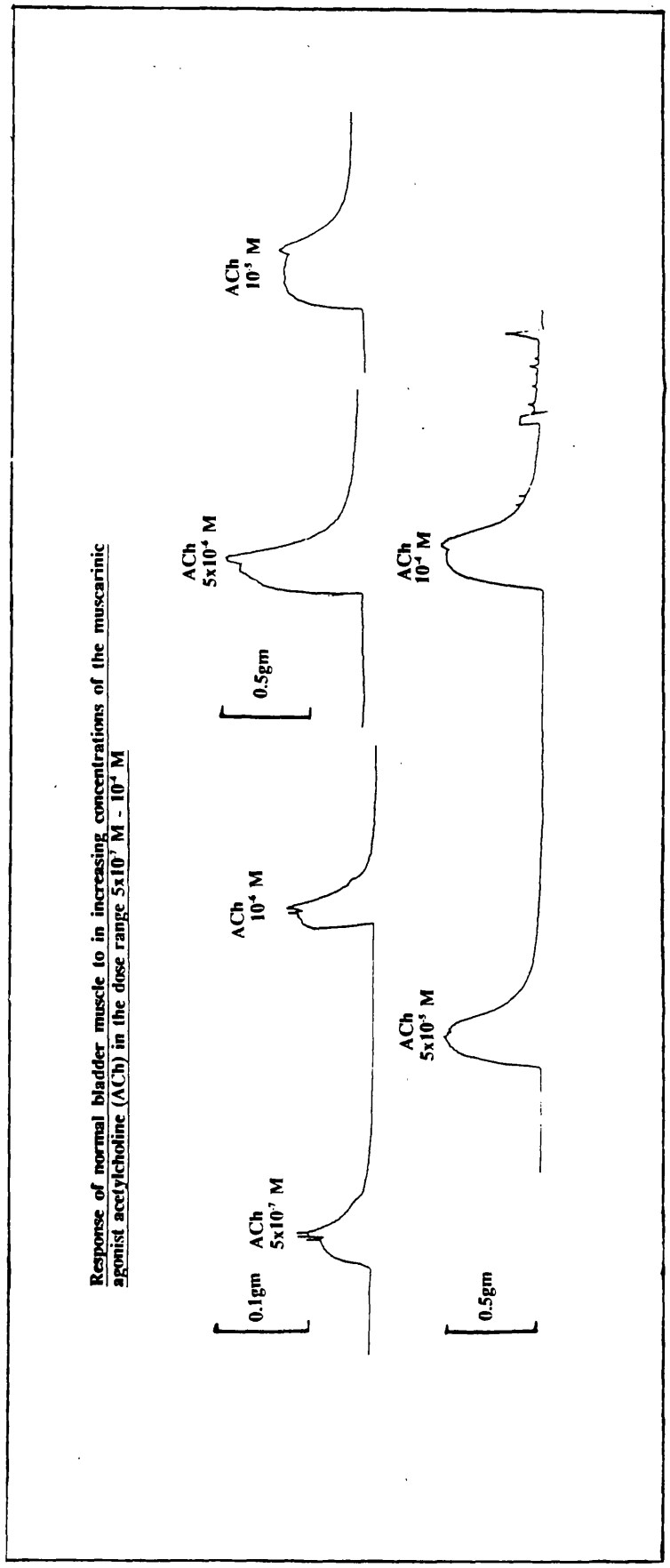

Response of normal bladder muscle to in increasing concentrations of the muscarinic agonist acetylcholine (ACh) in the dose range $5x10^{-7}$ M - 10^{-4} M
ACh $5x10^{-7}$ M
0.1gm
ACh 10^{-6} M
ACh $5x10^{-5}$ M
0.5gm
ACh $5x10^{-4}$ M
ACh 10^{-4} M
0.5gm
ACh 10^{-3} M

Figure 6.10:

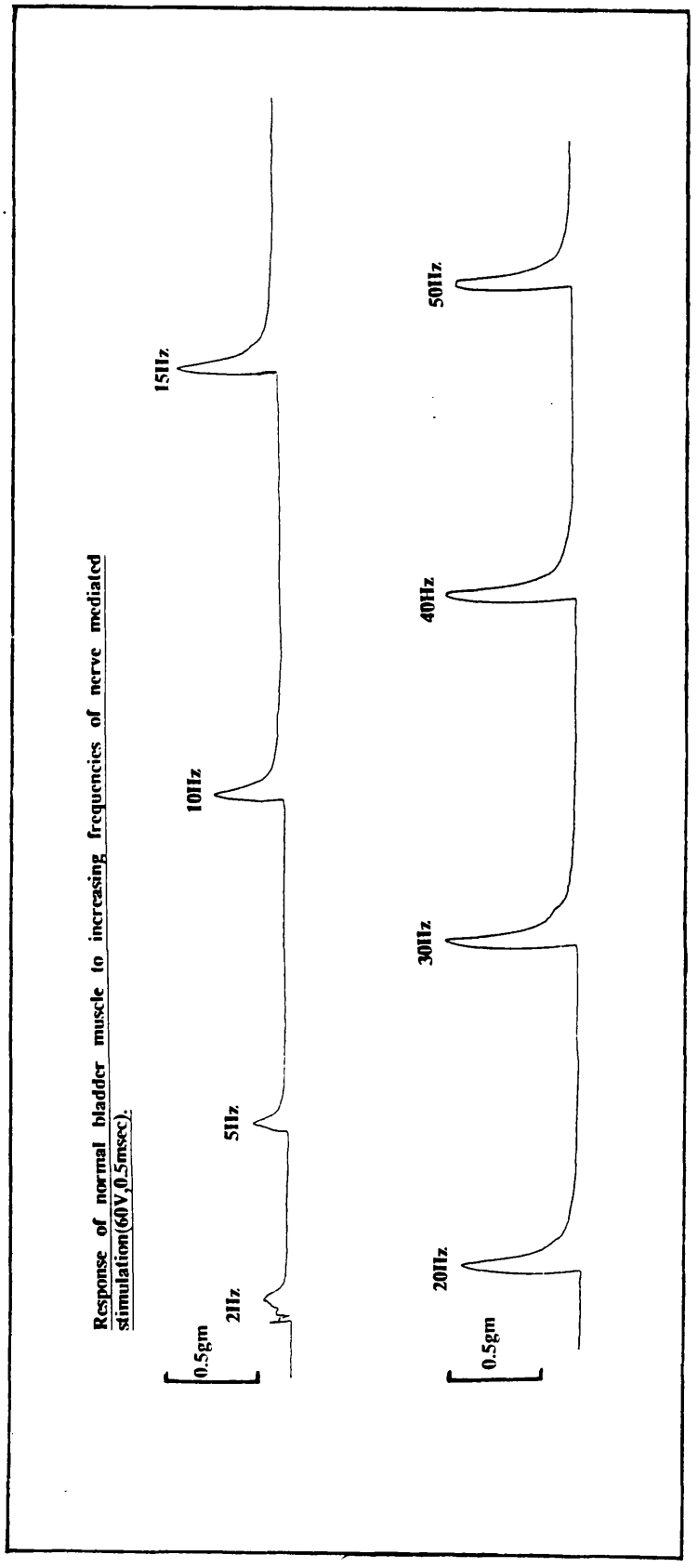
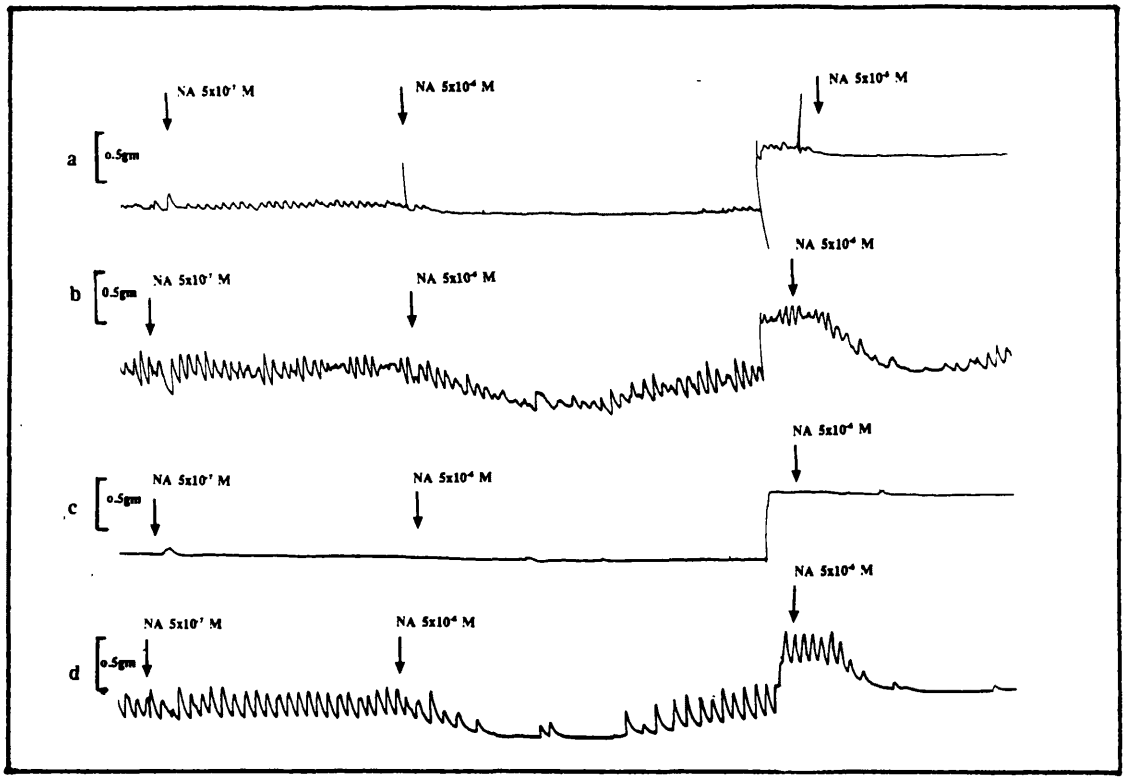
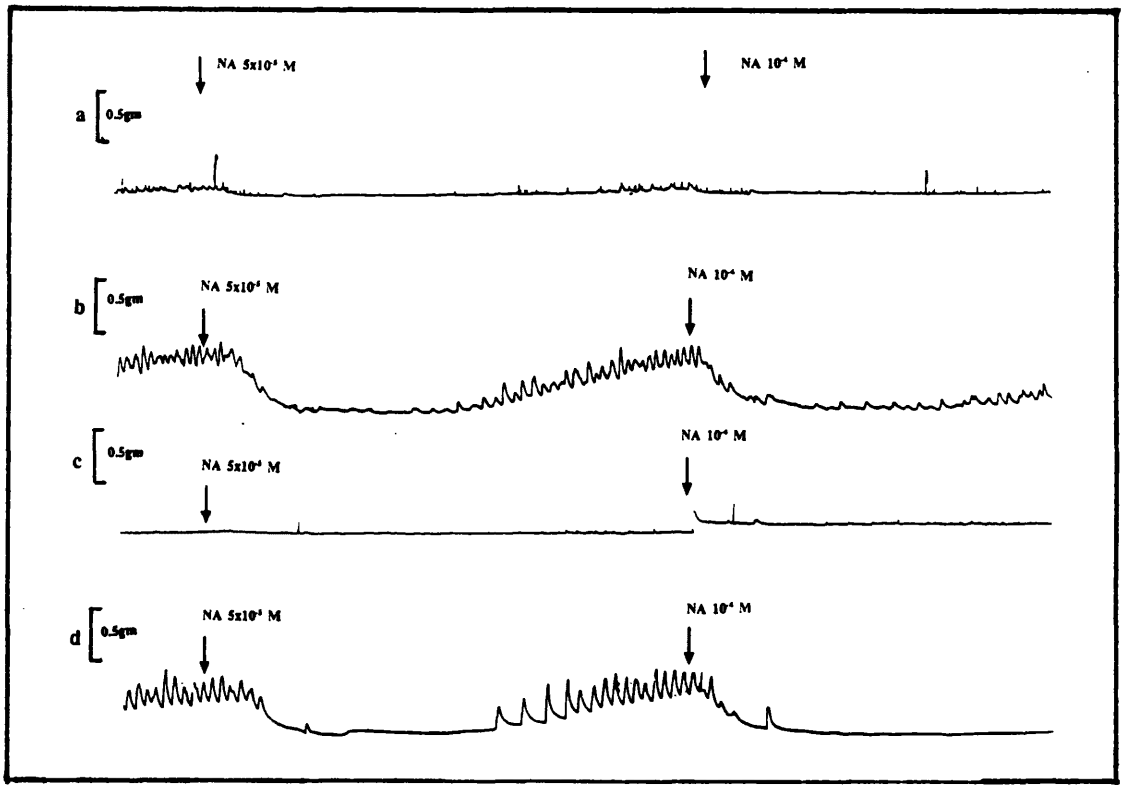

Response of normal bladder muscle to increasing frequencies of nerve mediated stimulation(60V,0.5msec).
0.5gm
2Hz
5Hz
10Hz
15Hz
20Hz
30Hz
40Hz
50Hz
0.5gm

Figure 5.14.





The varying responses of four normal bladder strip preparations (a-d)to the exogenous application of varying doses of noradrenaline (NA)$5x10^{-7}$ M to 10^{-3} M.This produced inconsistent response,with no demonstrable effect in two preparations (a) and (b) and relaxation in two preparations (c) and (d).

Figure 5.15.

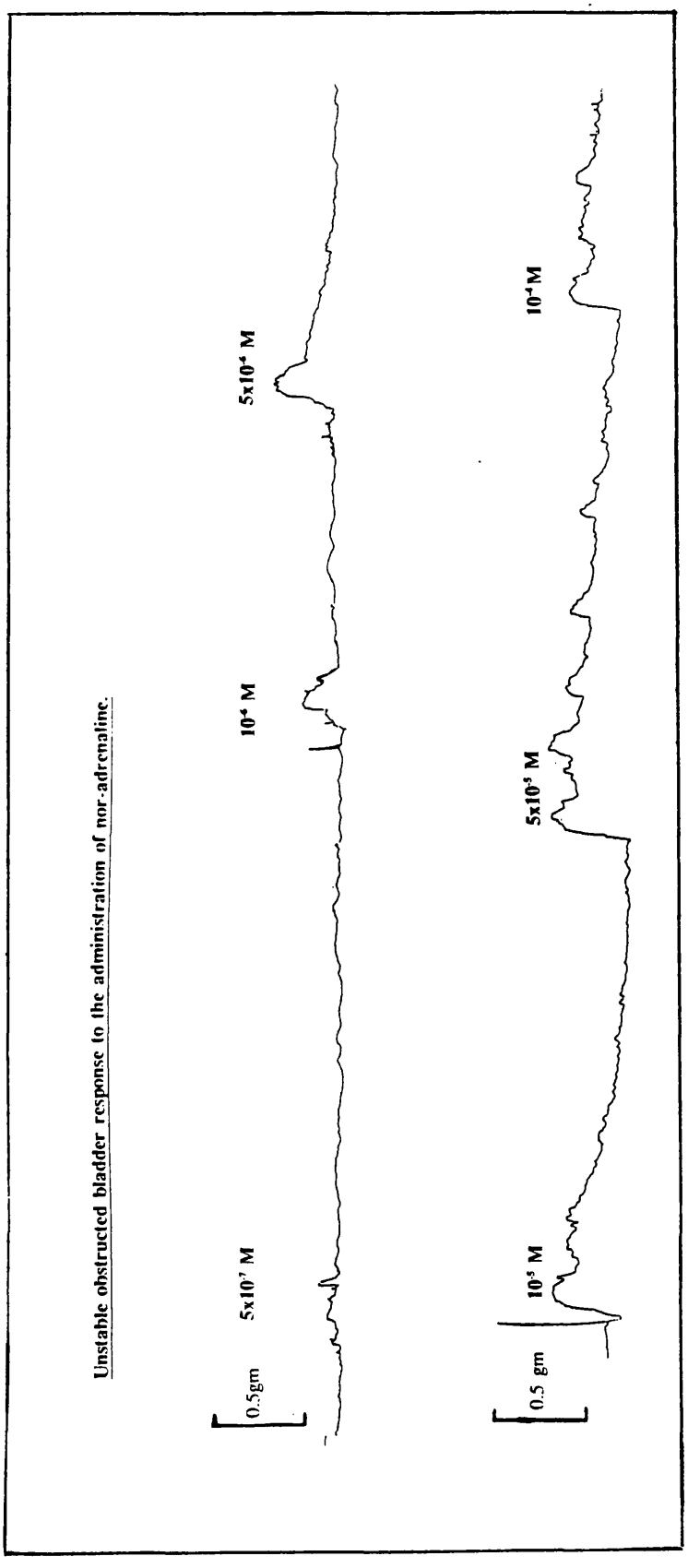

Unstable obstructed bladder response to the administration of nor-adrenaline.
0.5gm
5x10⁻⁷ M
10⁻⁶ M
5x10⁻⁶ M
0.5 gm
10⁻⁵ M
5x10⁻⁵ M
10⁻⁴ M

Figure 5.16.

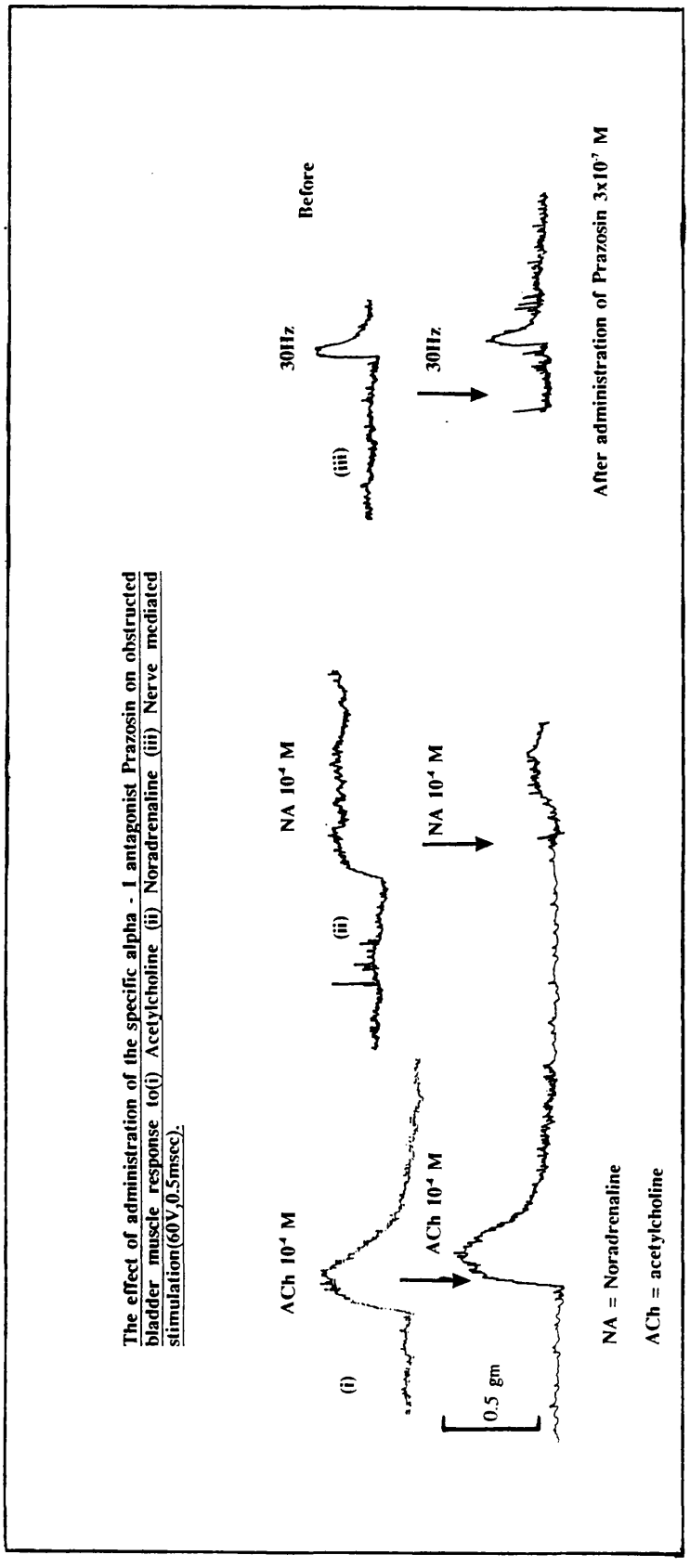
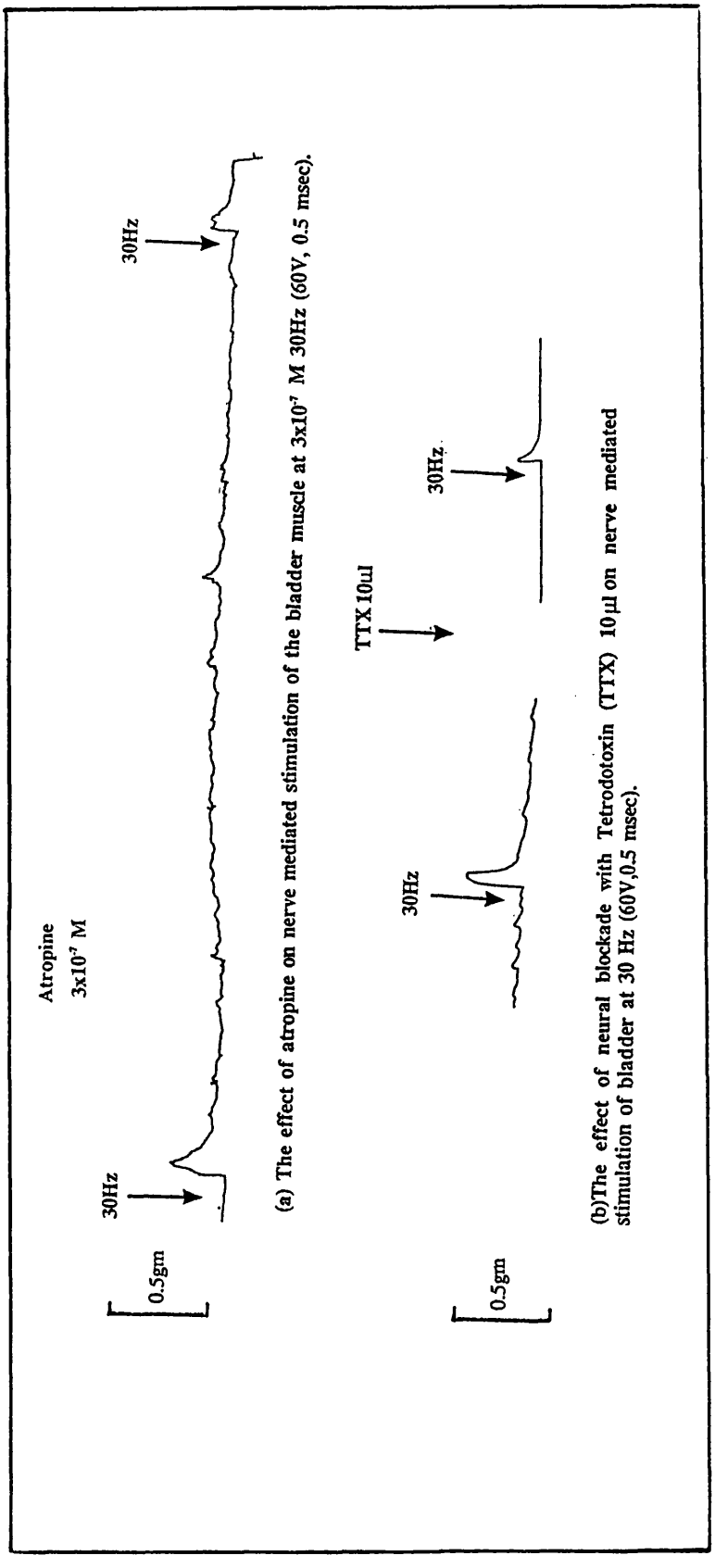

The effect of administration of the specific alpha - 1 antagonist Prazosin on obstructed bladder muscle response to(i) Acetylcholine (ii) Noradrenaline (iii) Nerve mediated stimulation(60V,0.5msec).
ACh 10⁻⁴ M
NA 10⁻⁴ M
30Hz
Before
(i)
(ii)
(iii)
ACh 10⁻⁴ M
NA 10⁻⁴ M
30Hz
0.5 gm
After administration of Prazosin 3x10⁻⁷ M
NA = Noradrenaline
ACh = acetylcholine

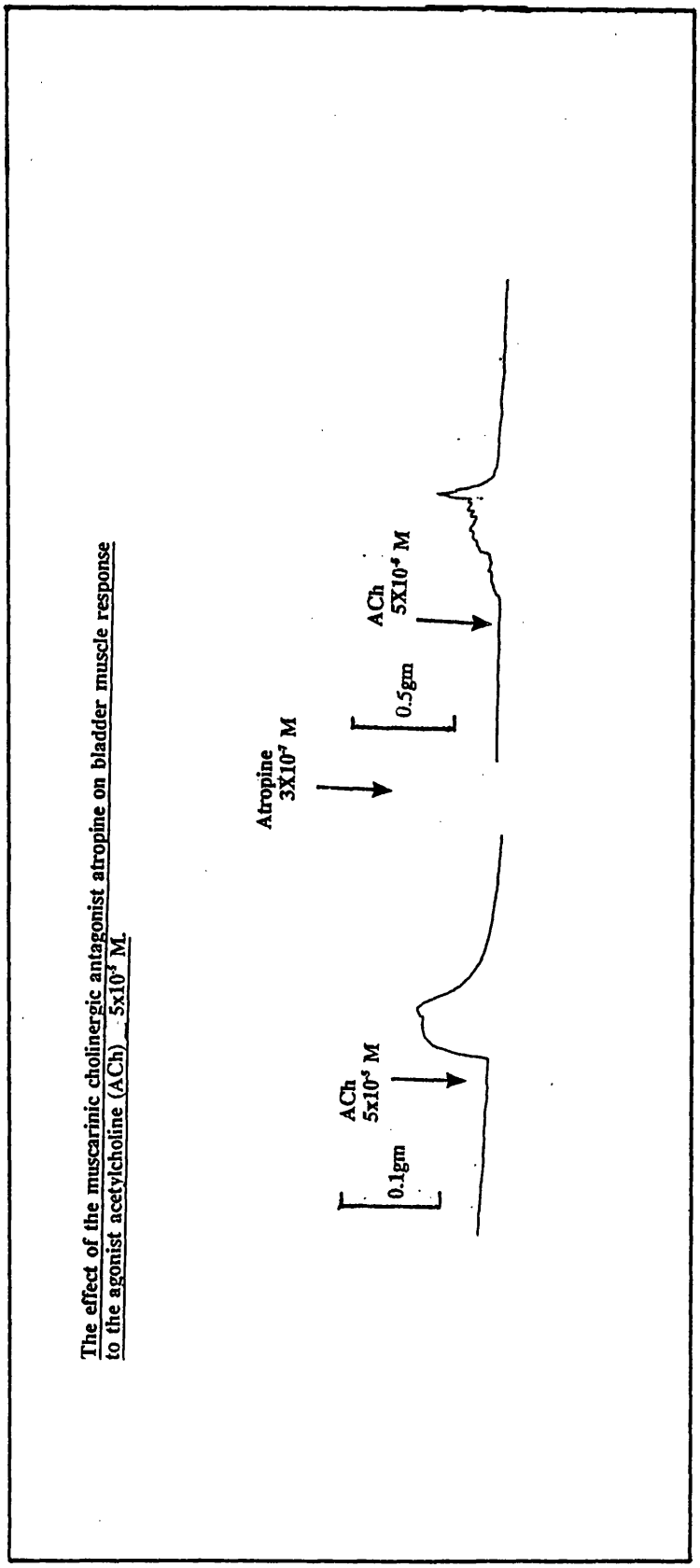
Figure 5.17:



(a) The effect of atropine on nerve mediated stimulation of the bladder muscle at $3x10^{-7}$ M 30Hz (60V, 0.5 msec).

(b)The effect of neural blockade with Tetrodotoxin (TTX) 10µl on nerve mediated stimulation of bladder at 30 Hz (60V,0.5 msec).

Figure 3.18:



The effect of the muscarinic cholinergic antagonist atropine on bladder muscle response to the agonist acetylcholine (ACh) $5x10^{-5}$ M.

Analysis of data using mean results for each patient studied (Figures 5.19, 5.20, 5.21)..

The following groups of patients were studied:- unstable obstruction n=6, stable obstruction n=3, control n=3, acute retention of urine n=4. The most striking observation resulting from the analysis of the mean data for individual patients was a consistent reduction in response of unstable obstructed detrusor to electrical stimulation (Figure 5.19), with a corresponding increase in response to the application of acetylcholine (Figure 5.20). However, neither of these changes reached statistical significance, when compared to the other groups studied. The other subgroups of obstructed bladder exhibited reduced responsiveness to neural stimulation as compared to control at lower frequencies, but the differences were again not statistically significant. However, the responses of unstable obstructed and stable obstructed bladder (5Hz,10Hz) as contrasted to acute retention bladder (30Hz-50Hz) were significantly different ($p<0.05$). The application of noradrenaline (Figure 5.21) produced a contraction of acute retention and unstable obstructed bladder, a change most marked in the former group which also exhibited a consistently reduced response to acetylcholine; again, none of these changes reached statistical significance.

Figure 5.19.

(Results expressed as a % of the maximal response to K^+)

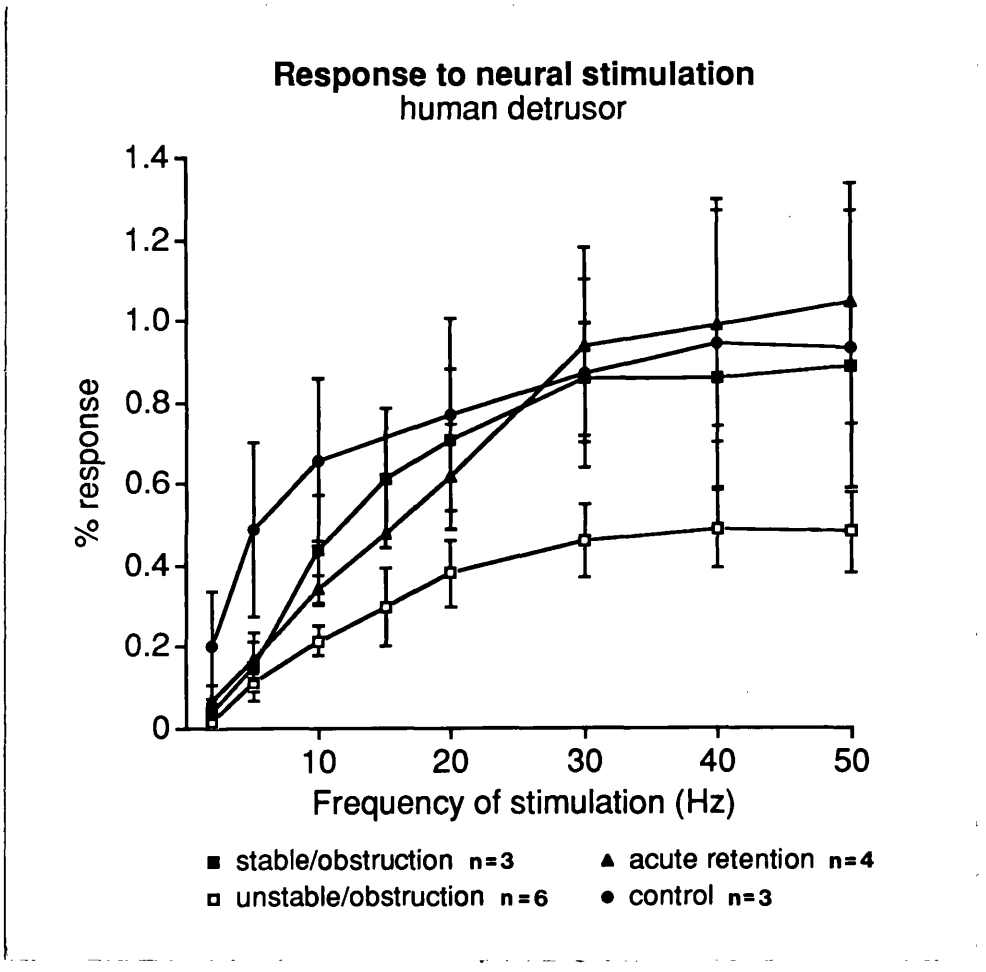


Figure 5.20.

(Results expressed as a % of the maximal response to K^+)

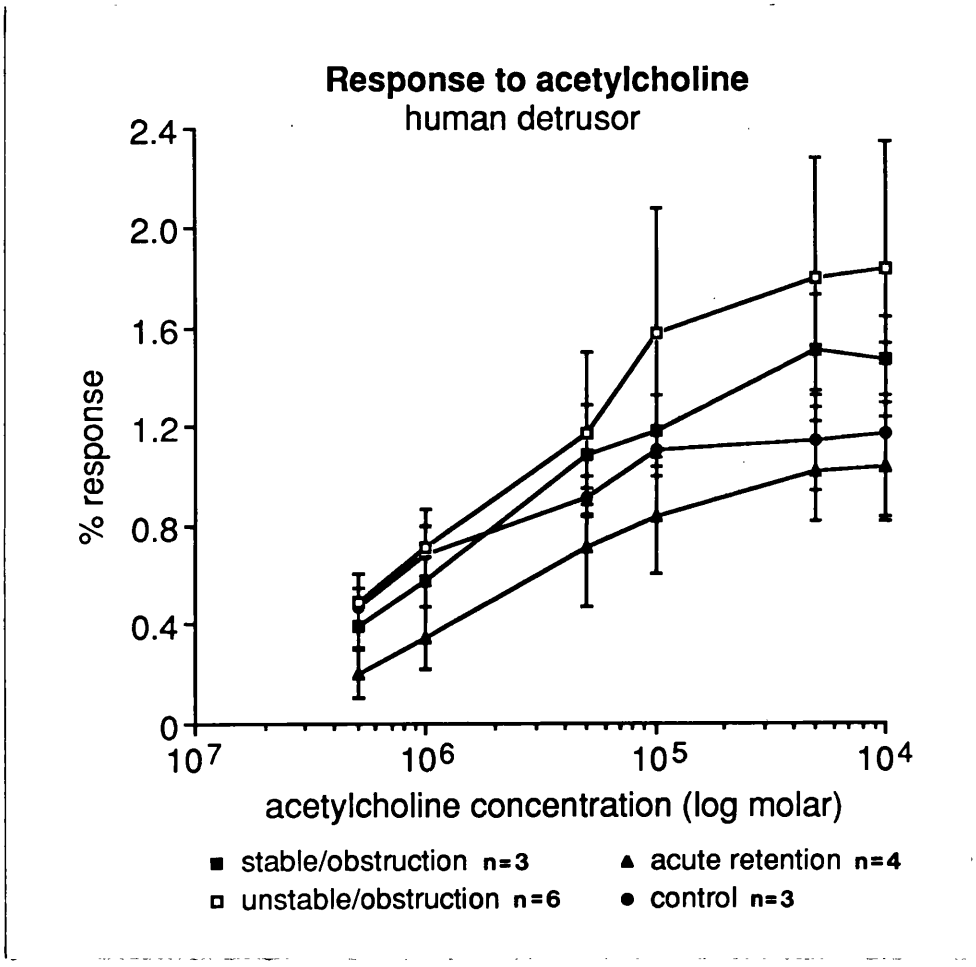
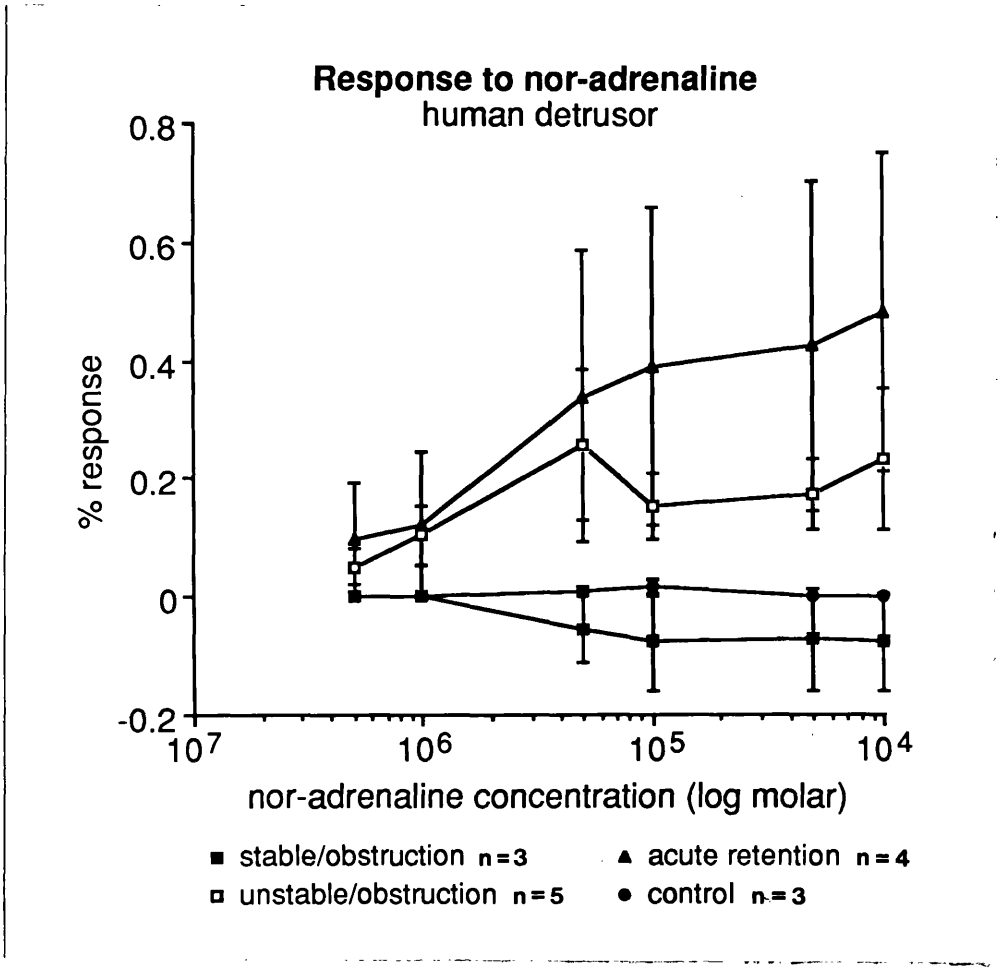


Figure 5.21.

(Results expressed as a % of the maximal response to K^+)



5.4 DISCUSSION

There is no doubt that major pathophysiological changes accompany the disruption of normal tissue architecture, as characterised by the collagen infiltration and muscle cell hyperplasia (Gilpin et al. 1985), which occur in the obstructed human bladder. The consequences of bladder outflow obstruction have been extensively studied in animal models, including the rat (Mattiasson, Uvelius 1982), pig (Sibley 1985), dog (Rohner 1978) and rabbit (Mayo and Hinman 1976, Levin et al. 1984,1987, Kato et al. 1988).

Although such studies have provided much information, it is nevertheless difficult to extrapolate the results to man because of inter-species variation. It must be remembered that although detrusor instability occurs in a majority of patients with bladder outflow obstruction, there is no quantifiable correlation between the severity of obstruction and the magnitude of instability (Turner-Warwick 1984). Furthermore, resolution of detrusor instability in the obstructed pig model often occurs without a corresponding change in in-vitro parameters (Sibley 1985, Speakman et al. 1987). Previous studies have considered the autonomic innervation of the bladder to be solely comprised of cholinergic or adrenergic nerves. It is now well recognised that a number of additional transmitters are involved (Burnstock 1986a,b); neuropeptide Y and adenosine triphosphate (ATP) as well as noradrenaline in sympathetic nerves, vasoactive intestinal polypeptide in addition to acetylcholine in parasympathetic nerves. Furthermore, sensory nerves containing substance P, calcitonin gene-related peptide and ATP have an

important influence on motor function (see Chapter 6).

A striking feature in this study was the pronounced reduction in AChE-positive nerves in all categories of obstructed bladder, most marked in the chronic retention bladder (89%). This confirmed the results of a previous study (Gosling et al. 1986) of male and female bladders, which documented a similar reduction in AChE-positive staining. It is of particular interest that the reduction in AChE-positive staining was similar in detrusor muscle from both stable and unstable bladders, a finding corroborated by work from a pig model (Speakman et al. 1987). In view of the absence of significant differences between stable, unstable and acute retention obstructed bladder it has to be concluded that these changes in the density of AChE-positive staining of nerves do not by themselves provide a satisfactory explanation for the altered detrusor function, seen in the obstructed bladder. Although it is tempting to speculate that the regression of the nerve count towards control values seen in the unobstructed post-prostatectomy group represents a reversal of previous damage, this was a small group and their bladders were unlikely to be normal since all of these patients were troubled by urinary frequency and two patients had detrusor instability. The markedly reduced innervation of the chronic retention bladder conforms with the clinical experience of detrusor failure in these patients. In interpreting these results it must be remembered that loss of AChE stained fibres does not necessarily reflect a loss of cholinergic excitatory nerves alone, but could also indicate a significant loss of sensorimotor (peptidergic) nerves (see Chapter 6).

Previous study of the normal human bladder has demonstrated a linear reduction with age in the density of AChE-positive nerves present within the bladder wall (Gilpin et al. 1986). No similar correlation between the neural count and age was evident in the present study, possibly related to the small number of control patients. Alternatively, it might be suggested that a factor other than age was influencing the results. Nevertheless, all patients within the study group were accurately age matched to a carefully selected control population. This is an important consideration since it seems likely that the prevalence of detrusor instability in the human male increases with age (Abrams 1985). Certainly, study of the rat highlights the important changes in bladder function which occur with increasing age (Chun et al. 1988); an observation supported by evidence of increased sensitivity of detrusor strips to acetylcholine and increased cholinoceptor density in older rats (Kolta et al. 1984).

The phenomenon of increased smooth muscle sensitivity to agonists is known as "supersensitivity" (Trendelenburg 1963). It is now well recognised that smooth muscle supersensitivity follows experimental damage to its motor innervation (Westfall 1981). Indeed such observations are not new, and formed the basis of Cannon's law which states that when an organ is deprived of its normal nerve supply it will develop hypersensitivity, in particular to its own neurotransmitter substance (Cannon 1949). Two qualitatively distinct manifestations of supersensitivity are recognised, termed prejunctional and postjunctional (Fleming et al. 1973). Prejunctional

supersensitivity only occurs if presynaptic mechanisms for the control of local agonist concentration are impaired, resulting in more acetylcholine being available at the smooth muscle cells. Postjunctional supersensitivity encompasses a situation where there is reduced contact between agonist and effector cell receptors, and includes situations such as an inhibition of transmitter release, receptor blockade and denervation.

It has been reported that the obstructed pig bladder (Sibley 1987) contracts more vigorously in response to cholinergic stimulation than normal tissue, with a corresponding reduction in response to nerve-mediated stimulation, findings which were attributed to a postjunctional cholinergic supersensitivity phenomenon. These results remain controversial, since further work using this model has reported that if the increased detrusor response to acetylcholine is related to the tissue mass then there is an overall reduction in the maximum force/gram tissue produced ((Speakman et al. 1987), confirming the observations of other workers (Levin et al. 1984, Susset et al. 1986)). In support of these observations, the maximum tissue contractile responses noted in this study upon stimulation of the obstructed bladder with potassium (Table 5.8) were considerably reduced ($p<0.05$) as compared to control.

The in vitro pharmacological results obtained in this study confirm that a disruption of normal detrusor muscle responsiveness to both pharmacological and nerve mediated stimulation occurs in the presence of bladder outflow obstruction. Although an inverse relationship was evident between

the responses to acetylcholine and electrical field stimulation in the unstable and stable obstructed groups, compatible with a post-denervation state, the differences were not statistically significant. These results only provide tenuous support for the hypothesis that post-junctional cholinoceptor super-sensitivity resulting from denervation is by itself a sufficient explanation for the development of detrusor instability in the obstructed bladder. The suggestion that denervation results in cholinoceptor supersensitivity has been challenged by Nilvebrant (1986). Using observations based on radio-ligand receptor binding studies and on measurements of affinity and receptor density, she concluded that muscarinic receptors in the rat urinary bladder are not involved in the development of supersensitivity.

Few previous studies of the responses of the obstructed as contrasted to control human bladder have been reported. Sibley (1985) documented a shift to the left of the dose response curve to acetylcholine at a dose range of $5x10^{-5}$ M up to $5x10^{-4}$ M and bladders from obstructed patients exhibited a significant decrease in the response to intramural nerve stimulation. Harrison et al. (1987) only demonstrated significant differences at stimulation frequencies of 30 to 50 Hz and at acetylcholine concentrations in the dose range 10^{-5} M - 10^{-4} M. In contrast, Eaton and Bates (1982) reported no differences in the responses of normal and unstable detrusor, but found an exaggerated response to electrical stimulation of muscle in the unstable group. This study equated normal with stable detrusor function and both groups included both male and female patients, the majority having

obstructed bladders (42/61).

The finding of a reduced response in acute retention bladder to cholinergic stimulation with little corresponding change in nerve-mediated stimulation cannot be explained by a denervation hypothesis. Alternative pathophysiological mechanisms therefore need to be considered. Administration of noradrenaline produced an α_1 mediated contractile response in the acute retention bladder, with a smaller but similar response in some preparations from unstable obstructed bladder. However, quantification of DBH-like immunoreactivity (albeit a relatively non-specific staining method for sympathetic nerves), failed to demonstrate any significant change in adrenergic innervation in the obstructed detrusor (Figure 5.9). In addition there was no evidence to suggest an increase in adrenergic nerve density similar to that reported in the decentralised human bladder (Sundin et al. 1977). Isometric muscle strip studies demonstrated that prazosin did not antagonise nerve mediated contraction of detrusor muscle, thereby suggesting that the altered adrenergic response seen in the obstructed bladder resulted from changes in receptor function rather than nerve density. The significant increase in noradrenaline content seen in the unstable obstructed bladder body (Figure 5.10) is difficult to explain satisfactorily on the basis of current evidence. Unfortunately due to the limited tissue available, further bio-chemical assays were not possible in the other obstructed subgroups.

Previous experimental studies of adrenoceptor stimulation in the obstructed bladder have produced conflicting results, with documented

relaxation in pig bladder (Sibley 1987) and contractile responses in both dogs (Rohner et al. 1978) and humans (Perlberg and Caine 1982). The work reported here confirms the observations of Perlberg and Caine demonstrating a correlation between α_1 adrenoceptor-mediated contractile responses and detrusor instability. The observation of a prominent α_1 adrenoceptor mediated contractile response in the acute retention bladder presented here has not previously been reported. While this observation is only based on the study of a small number of specimens, an attractive hypothesis is that the altered tissue sensitivity to adrenergic stimulation could explain the unpredictable tendency for a subgroup of patients to develop acute retention in situations of increased sympathetic stimulation. The resultant episode of retention could then be equated to the detrusor-sphincter dyssynergia seen in patients with primary neuropathic disorders.

The potential mechanism of altered adrenoceptor function remains obscure. Certainly changes in receptor function are well recognised elsewhere in the body in response to alterations in parameters such as the degree of stretch of muscle fibres (Benson et al. 1975) and hormonal milieu (Levin et al. 1981, Anderson and Navarro 1988). The inconsistent and relatively limited contractions seen in response to the application of noradrenaline in detrusor muscle from unstable obstructed patients suggests that although it may be a contributory factor it is by itself insufficient to explain the pathogenesis of secondary detrusor instability.

Whilst there is good evidence for a disruption of neural mechanisms, the

mechanism of injury to the nerves remains speculative. Raised intravesical pressure could lead to a reduction in blood flow to detrusor muscle, which indeed has been shown to occur in experimental models at surprisingly low intraluminal pressures (Dunn 1974). Subsequent ischaemia would alter the structural characteristics of both smooth muscle cells and nerve fibres. Such an injury could compromise the function of the membrane bound Na^+ pumps, which require a constant supply of high energy phosphate and which would be particularly susceptible to such damage (Sehn 1979).

Since none of the neuropathic hypotheses have fully explained the aetiology of detrusor instability, it is likely that there may be an additional functional abnormality of the obstructed detrusor smooth muscle (Malmgren et al. 1987). Certainly, marked structural changes within the human detrusor muscle follow obstruction (Uvelius et al. 1989) and this study demonstrated a reduced maximal contractile response of obstructed human detrusor to high dose potassium, confirming previous animal studies (Levin et al. 1984, Uvelius et al. 1988).

Experimental study of denervated smooth muscle has revealed important neurophysiological changes rendering the cell more susceptible to stimuli (Fleming and Westfall 1975). Westfall (1981) demonstrated partial depolarisation of the muscle cell membrane following chronic denervation of the guinea pig vas deferens. If similar changes occur in the obstructed human detrusor this would tend to increase the sensitivity of the smooth muscle cell to any agonist which induces contraction by depolarising the cell membrane,

thereby non-specifically increasing responsiveness to stimuli unrelated to the naturally occurring neurotransmitter. Post-junctional supersensitivity arising from a disruption of cholinergic innervation, altered adrenergic neural control, an abnormality of normal inhibitory mechanisms (Kinder and Mundy 1985) and altered local spinal reflex activity (Chalfin and Bradley 1982, Steers and deGroat 1988) could all lead to changes in the physiological responses of smooth muscle cells.

Potentially, such detrusor muscle supersensitivity could facilitate the spread of waves of contraction throughout the detrusor muscle. Support for this suggestion is provided by the in vitro demonstration of exaggerated responsiveness of obstructed as compared to control bladder upon direct electrical stimulation of the pig (Sibley 1985) and rat (Malmgren et al. 1988) detrusor muscle. Indeed, detrusor electromyography has demonstrated an abnormally hyperactive pattern of voltage fluctuation in the detrusor muscle of patients with primary detrusor instability (Doyle and Hill 1976).

Bladder outflow obstruction leads to marked changes in the function of the human bladder with concomitant abnormalities seen in both ultra-structural and in vitro neuropharmacological studies. The results discussed here suggest the interplay and summation of a number of different patho-physiological mechanisms and support the view that a global disruption of neural function accompanies the marked ultrastructural changes seen in the obstructed detrusor muscle. These observations lend credence to the suggestion that both drugs which stabilise muscle cell membranes (Malmgren

et al. 1989b) and α_1 antagonists (Chapple et al. 1989) may have a potential therapeutic role in the treatment of obstructive detrusor instability. Further studies of obstructed human bladder are necessary to investigate the role of changes in receptor density, affinity and distribution, agonist release and degradation and the ultrastructural and physiological alterations following the relief of obstruction. An area which remains little explained is the role of the afferent innervation of the bladder and in particular, the effect of obstruction on putative non-adrenergic, non-cholinergic neurotransmission.

CHAPTER 6

CHANGES IN THE NON-ADRENERGIC NON-CHOLINERGIC SENSORIMOTOR INNERVATION OF THE OBSTRUCTED HUMAN BLADDER

6.1 Introduction

The human bladder body receives a dense uniform parasympathetic innervation comprised predominantly of cholinergic nerves (Gosling et al 1977), in contrast to the sympathetic adrenergic innervation which is sparse and non-uniform (Sundin et al 1977, Benson et al 1979). Co-ordinated contraction of the urinary bladder at the time of micturition is initiated and maintained by parasympathetic nervous stimulation via the pelvic nerves in man and all mammalian species studied (Andersson and Sjogren 1982). Conversely there is no evidence to support a significant primary role for the sympathetic nervous system in the control of human bladder filling (Awad et al. 1974, Jensen 1981, Cowan and Daniel 1988). Langley and Anderson (1895a) first reported partial atropine-resistance during electrical stimulation of the sacral roots to the dog bladder; however it was not until the early 1970's that the concept of non-adrenergic non-cholinergic (NANC) neuro-transmission in the bladder was introduced (Ambache and Aboo Zar 1970, Burnstock 1972, Taira 1972). It is widely accepted that adenosine 5'-triphosphate (ATP) is the neurotransmitter utilised by NANC excitatory nerves supplying the mammalian urinary bladder (Burnstock 1972, Burnstock et al 1978), and autoradiographic analysis demonstrates a high

specific binding for purinoceptors in the rat bladder (Bo and Burnstock 1990). Indeed, recent in vitro pharmacological studies have demonstrated a small purinergic component in the human bladder in response to electrical field stimulation (Hoyle, Chapple et al 1990). Although a matter of debate (Cowan and Daniel 1983, Husted et al 1983) as to whether the atropine-resistant TTX-sensitive component plays a significant physiological role in normal human detrusor function (Sibley 1984b), it seems more likely that NANC neuro-transmission may be of greater importance in the functionally disturbed bladder (Sjogren et al. 1982).

It is well recognised that the human bladder contains several neuropeptides including vasoactive intestinal polypeptide (VIP), substance P (SP), somatostatin (Som) and neuropeptide Y (NPY); (Larsson et al 1977, Gu et al 1984, 1983b and Crowe et al 1990). This study was designed to investigate the normal histological distribution of the putative neurotransmitter peptides neuropeptide Y, vasoactive intestinal polypeptide, substance P, calcitonin gene related peptide (CGRP) and somatostatin which are thought to play an important role in sensorimotor nerves and to document the changes that occur in association with bladder outflow tract obstruction.

6.2 Methods

6.2.1 PATIENTS

Bladder tissue was obtained at the time of prostatectomy from the

lateral walls of the bladder dome below the peritoneal reflection. The study population comprised patients undergoing surgery for the relief of benign prostatic outflow obstruction and in whom there was no evidence of renal dysfunction or co-existing prostatic neoplasia. All patients underwent full preoperative urodynamic assessment using the technique of videocystometry (Bates et al. 1970), thereby allowing pre-operative subdivision of separate groups (see Chapter 5).

The groups submitted to histological analysis were as follows:- Stable obstruction n=5, mean age 65.80±2.65 years; unstable obstruction n=9, mean age 67.44±2.69 years; acute retention n=5, mean age 69.40±3.49 years. In addition, tissue was obtained from the bladders of unobstructed patients undergoing other surgical procedures. These patients had normal flow rates and there was no clinical or histological evidence of bladder outflow obstruction (n=4, mean age 56±7.78 years). Although the mean age of the control population was younger than the obstructed patients, the difference was not statistically significant at the 5% level.

The groups submitted for biochemical assay were as follows:- Stable obstruction n=3, mean age 64.79±2.34 years; unstable obstruction n=6, mean age 65.32±2.21years; acute retention n=6, mean age 68.71±3.58 years; control n=4, mean age 56±7.78 years.

6.2.2 TISSUE PREPARATION FOR HISTOLOGICAL ANALYSES

Each biopsy specimen was immediately fixed to a small piece of cork using the tissue mounting solution "Tissuetek".

i) Some of the tissue was then immediately frozen in theatre in isopentane (2-methylbutane) which had been previously cooled directly in liquid nitrogen to -160 ℃. The frozen tissue was then carefully labelled and preserved in a -70℃ store. The frozen tissue was transported to laboratories either packed on dry ice or in liquid nitrogen and again stored at -70℃. In the laboratory, the material was removed from the -70 ℃ refrigerator and allowed to equilibrate for 5 minutes in a -20℃ cryostat. Serial sections were cut from each tissue block with a microtome at thicknesses of 10-20 µm. Tissue was sectioned and stained for routine histology using Masson's trichrome (1929).

ii) Other tissue collected for specific immunohistochemistry was fixed rapidly in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.10-7.4 for 1.5 h at room temperature, rinsed in PBS containing 7% sucrose and 0.1% sodium azide, and left overnight at 4°C in the same buffer.

6.2.3 HISTOLOGICAL TECHNIQUES

A. Routine Histology.

Masson's trichrome technique (1929) was used (see Chapter 5.2.3).

B. Immunohistochemistry.

The tissue sections (10 µm) were cut on a cryostat and immuno-fluorescence staining was carried out using the indirect method (Coons et al. 1955). The sections were incubated for 18 hours at room temperature with antisera raised in rabbits to neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), substance P (SP), calcitonin gene-related peptide (CGRP)

and somatostatin (SOM) (all from RIA, U.K.) at a dilution of 1:200 (NPY), 1:1000 (VIP) and 1:200 for the remaining antisera, then rinsed in PBS and incubated for 1 h at room temperature with fluorescein isothiocyanate conjugated sheep anti-rabbit immunoglobulin (Nordic) at a dilution of 1:100. After a further PBS wash, the sections were mounted in PBS/glycerol (1:1) and examined using a Zeiss fluorescence microscope fitted with epi-illumination. Control sections were incubated with either normal rabbit serum, or the antiserum absorbed with the substance under investigation.

C. Quantitative Methods for Light Microscopy

All sections were coded prior to processing and staining and were examined by the observer in a blind fashion.

Semiquantitative assessment. Sections were coded and subjectively quantified, for the density of immunoreactive nerves observed on an arbitrary scale of 1 to 5 (1 = sparse and 5 = dense).

Quantitative assessment. Full details of the technique used are to found in chapter 5.2.4e. The objective technique used is illustrated below (Figure 6.1).

6.2.4 NEUROCHEMISTRY

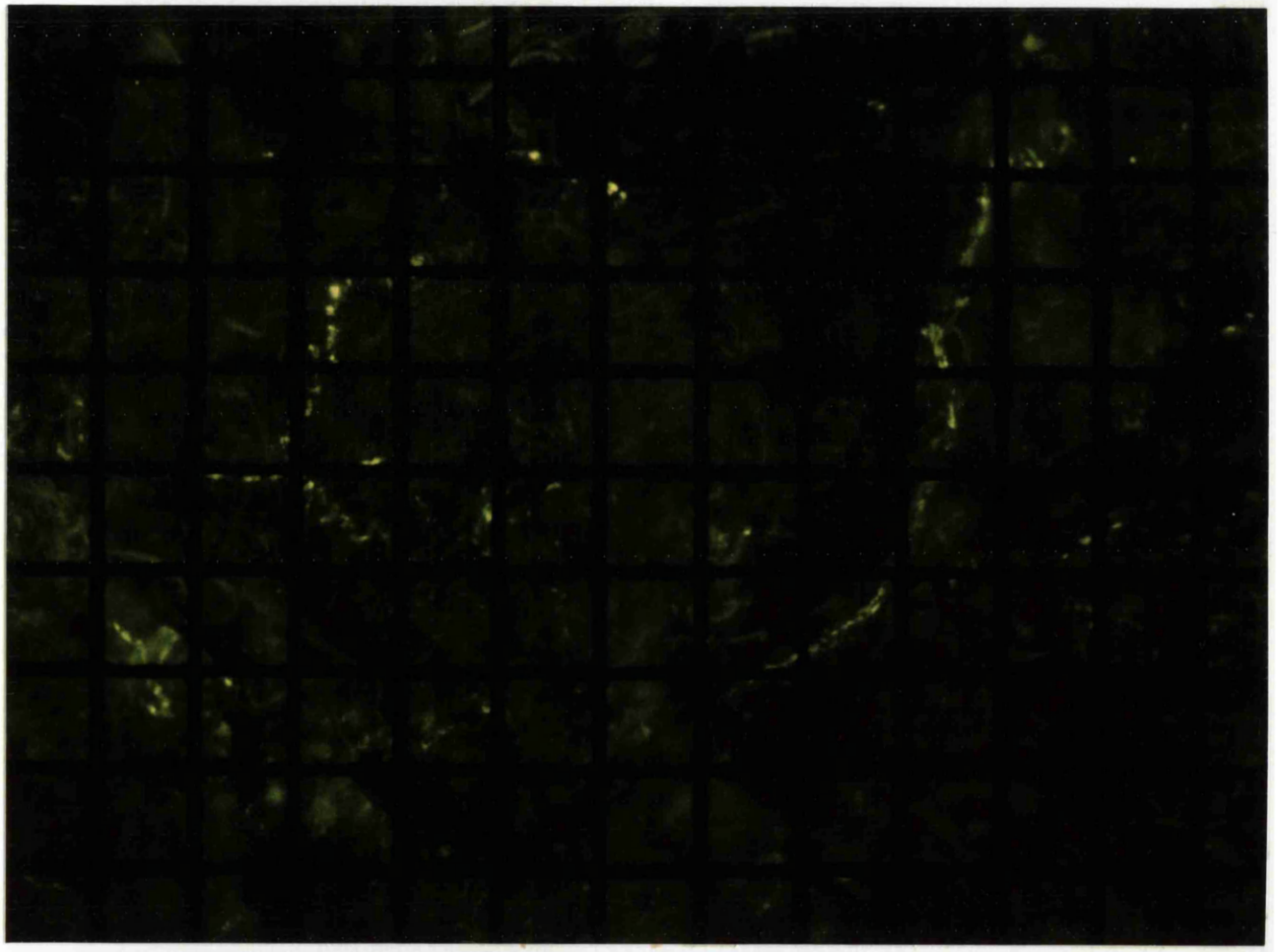
Peptide Assay

Pieces of tissue were stored as described above in liquid nitrogen until peptide extraction. The bladder tissue was weighed and the peptides extracted into 0.5 M acetic acid in polypropylene tubes in a boiling water bath for 15 min. The samples were homogenized, centrifuged for 30 min at 3500 g and lyophilized.

Vasoactive intestinal polypeptide (VIP), substance P (SP), calcitonin gene related peptide (CGRP) and neuropeptide Y (NPY) were quantified using an inhibition enzyme-linked immunosorbent assay as described previously (Belai et al. 1985, Stjernschantz et al. 1982) and the results expressed as pmol/g tissue. For each incubation, flat-bottomed polystyrene microtitre plates (Dynatech Laboratories Inc., Alexandria, VA), each containing 100µl of solution were coated with VIP (0.1µg/ml), SP conjugated to poly D-glutamate (0.5µg/ml), CGRP(0.1µg/ml) and NPY (0.15µg/ml); in 0.1 M carbonate-bicarbonate buffer, pH 9.6, containing 0.02% sodium azide, by incubating for 18-24h at 4°C. The contents of the plates were discarded and washed three times with PBS/Tween and incubated for 1h at room temperature with PBS/Tween containing 0.1% gelatine to prevent non-specific binding. After emptying the plates by inversion, extracted samples which had been reconstituted in PBS/Tween containing 0.1% gelatine, 0.02% sodium azide and 0.001% aprotinin (Sigma Chemicals Co., Poole, UK) at 0°C, and standards (50µl) were added to each well followed by 50µl of antiserum raised in rabbits to synthetic VIP, CGRP, substance P and NPY diluted 1:7500 for VIP and substance P and 1:12500 for CGRP and NPY in sample buffer. The plates were covered and incubated for 3 days at 4°C. The plates were then washed three times with PBS/Tween and 100µl of alkaline phosphatase conjugated goat anti-rabbit immunoglobulin (Sigma Chemical Co., Poole, U.K.) was added to each well at a dilution of 1:500 in sample buffer. The plates were incubated in a humid chamber for 2h at 37°C. The

Figure 6.1.

The graticule technique applied to a section of human bladder showing nerves within the detrusor muscle exhibiting VIP-immunoreactivity (magnification x100, graticule not to scale).



unbound goat anti-rabbit immunoglobulin conjugated to alkaline phosphatase was removed using three washes with PBS/Tween and one wash with glycine buffer, containing 0.001 M magnesium chloride and 0.001 M zinc chloride (pH 10.4). The chromogenic substrate p-nitrophenyl phosphate, 1 mg/ml in glycine buffer, was added to each well and the colour allowed to develop at room temperature. The absorbence was read in a Titertek Multiscan automatic spectrophotometer (Flow Laboratories, Rickmansworth, Herts, U.K.) at 405nm.

6.2.5 STATISTICAL ANALYSIS

All results are expressed as mean ± S.E.M. Statistical analyses were performed to contrast patients with controls and compare results obtained in each sub-group where appropriate. Although in most groups the data approximated to a normal distribution, in view of the small sample sizes non-parametric statistical analysis was performed using the Mann Whitney U test to determine statistical significance. Data analyses were carried out with the aid of the Minitab 5.1.1 software package on a Tandon IBM-compatible computer. A level of probability of 0.05 or less was considered significant.

6.3 RESULTS

6.3.1 Histological Localisation of Neuropeptides

The highest density of neural immunoreactivity in the bladder was to NPY. Although nerve staining for this compound was occasionally seen in the submucosal tissue and around blood vessels (Figure 6.2), its predominant localisation was in the detrusor muscle layer (Figure 6.3). VIP was present with a similar distribution (Figure 6.4). In addition a number of VIP-like immunoreactive nerves were present within the lamina propria and to a lesser extent the submucosa. Both VIP and NPY-like immunoreactivity was also equally evident in association with blood vessels. No immunoreactive neural ganglia were identified. CGRP-like immunoreactivity was found predominantly in relation to vessels with only isolated nerve fibres being identified in detrusor muscle and submucosal tissues (see figure 6.5). SP-like immunoreactive nerves, although present in a similar density to CGRP, were principally localised to the mucosa and submucosal layers. In contrast, there were very few SOM-like immunoreactive nerves and when present these were localised within the submucosal tissues.

6.3.2 Neural Quantification

Semi-quantitative assessment suggested there was a significant reduction in immunoreactivity to neuropeptides in the obstructed bladder (see Figures 6.6b, 6.7b, 6.8b, 6.9b, 6.10b). Use of an objective technique as previously described by Gosling (1986), with correction of the results for muscular hypertrophy and alterations in the connective tissue content of detrusor

Figure 6.2

NPY-immunoreactivity in a section of human bladder demonstrating nerves associated with blood vessels in the submucosal layer (magnification x90).

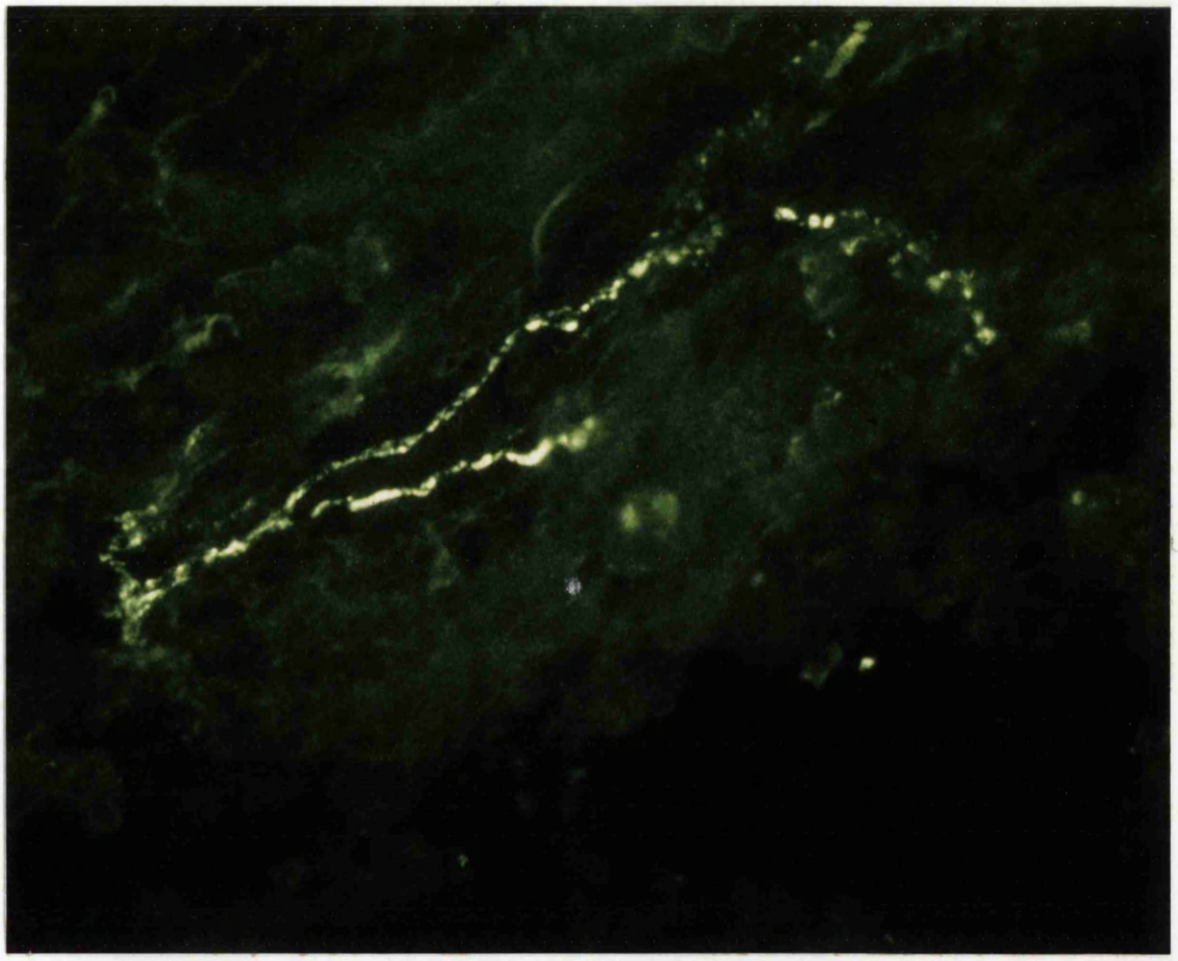


Figure 6.3

NPY-immunoreactivity within a section of human bladder demonstrating nerves within the detrusor muscle (magnification x90).

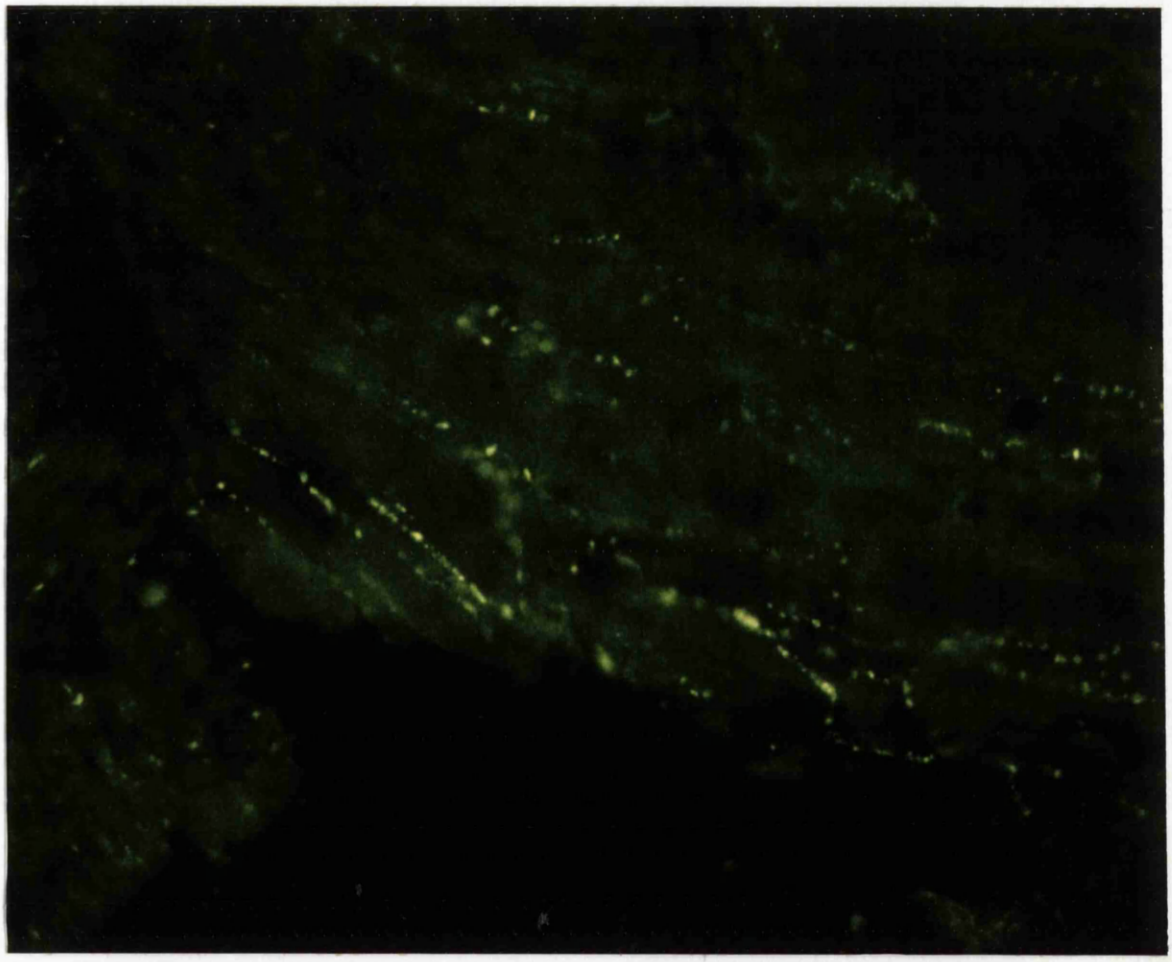


Figure 6.4

VIP-immunoreactivitv within a section of human bladder demonstrating nerves within the detrusor muscle (magnification x90).

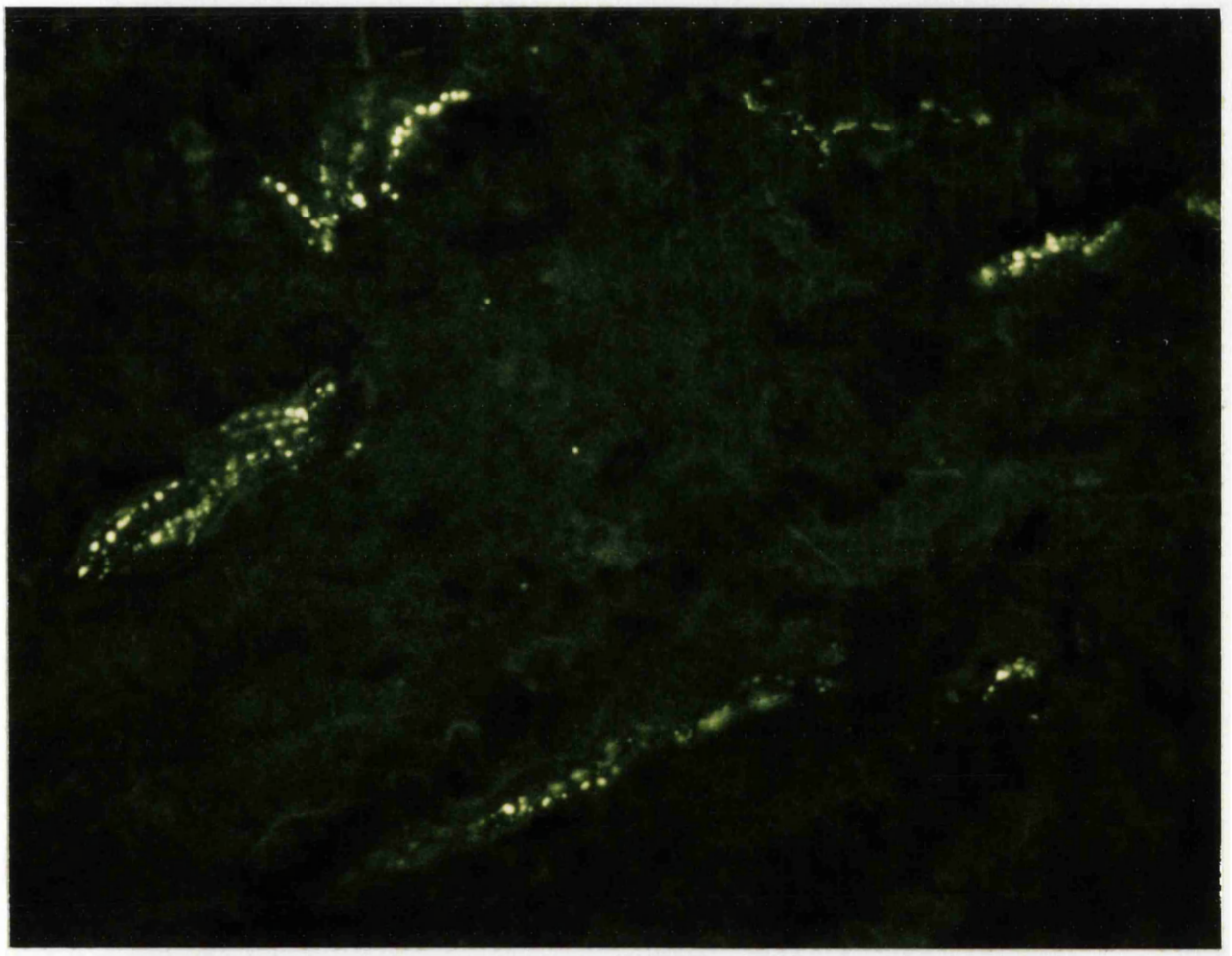


Figure 6.5

CGRP-immunoreactivity within a section of human bladder demonstrating immunoreactivity within bladder submucosa associated with blood vessels, in adjacent sections from the same patient. a-c (calibration bar=25μm).

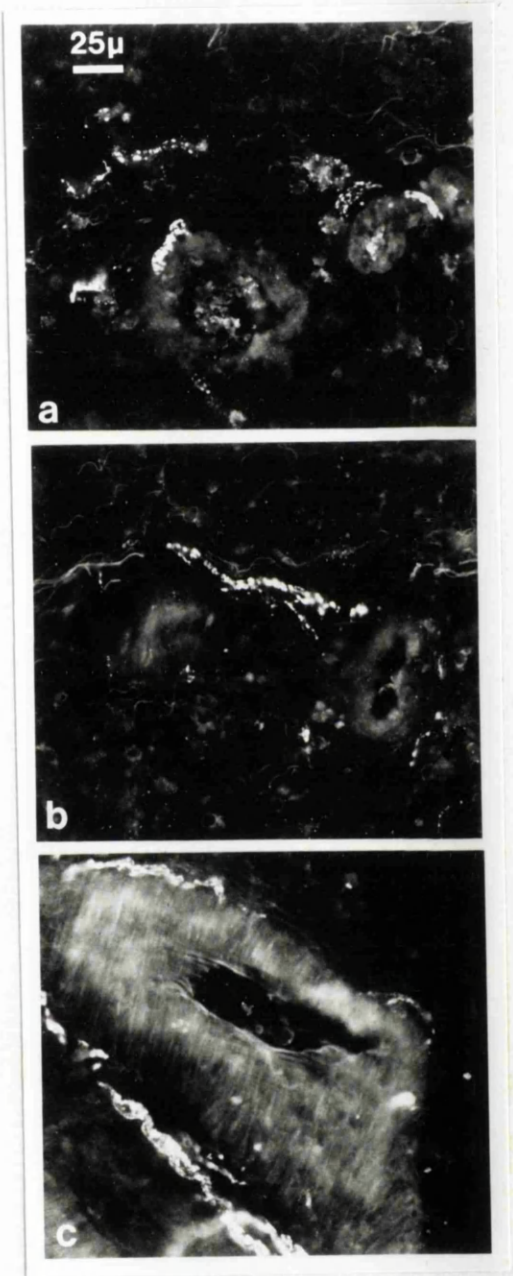
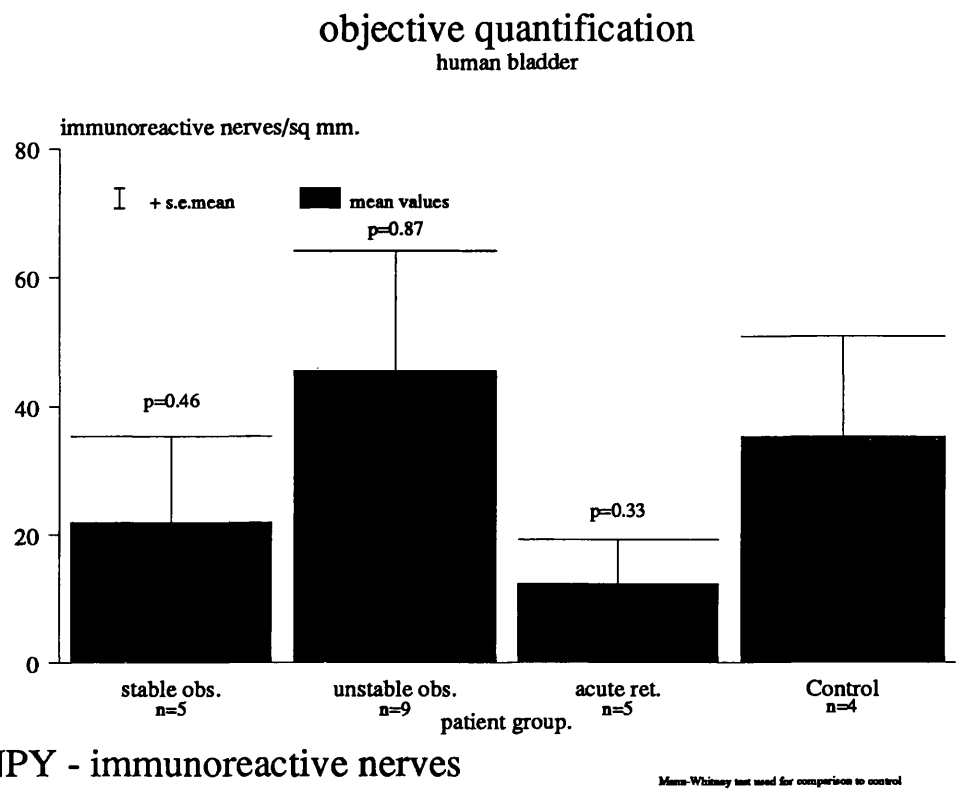


Figure 6.6

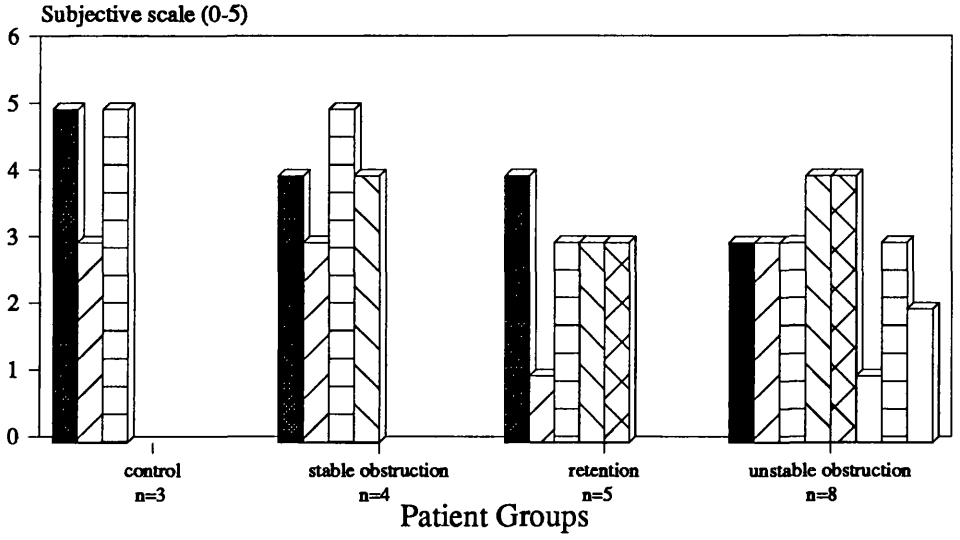
a)



NPY - immunoreactive nerves

Mann-Whitney test used for comparison to control

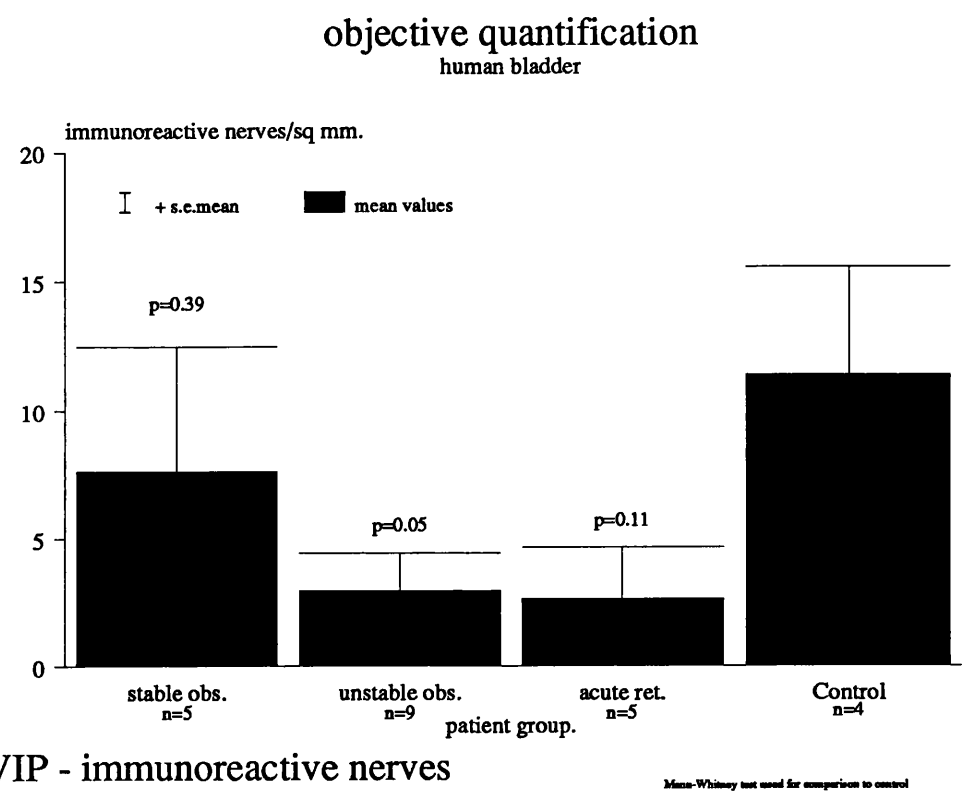
b)



NPY - IMMUNOREACTIVE NERVES

Figure 6.7.

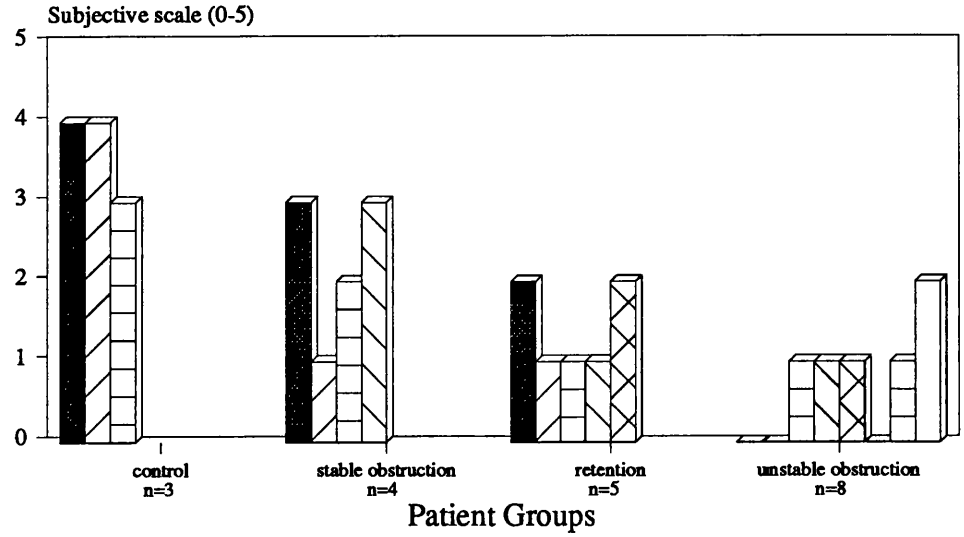
a)



VIP - immunoreactive nerves

Mann-Whitney test used for comparison to control

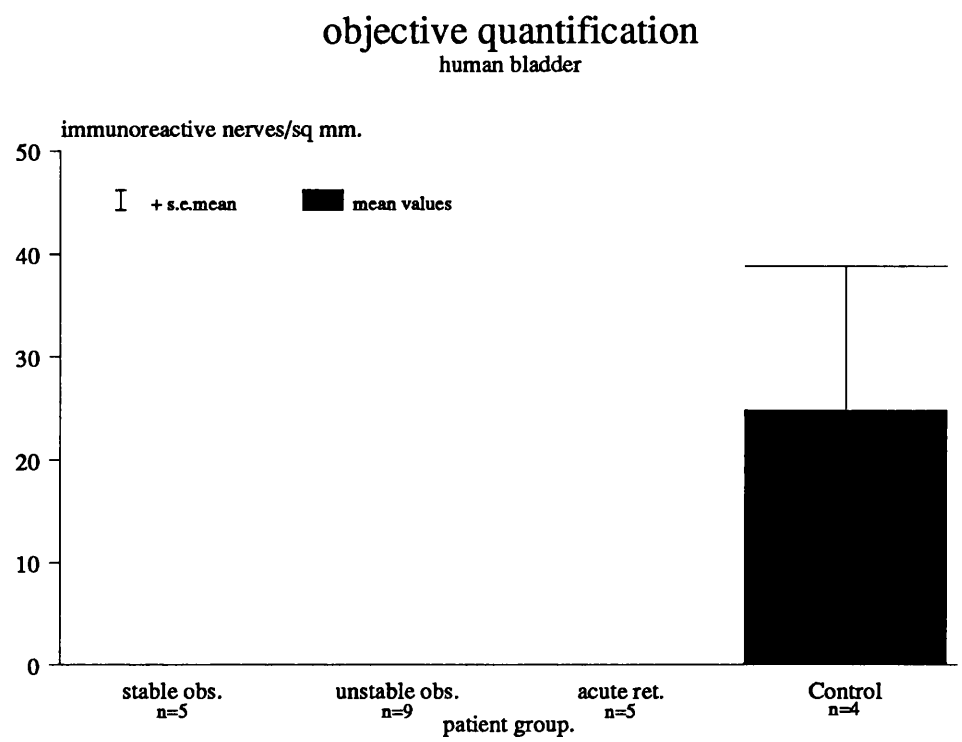
b)



VIP - IMMUNOREACTIVE NERVES

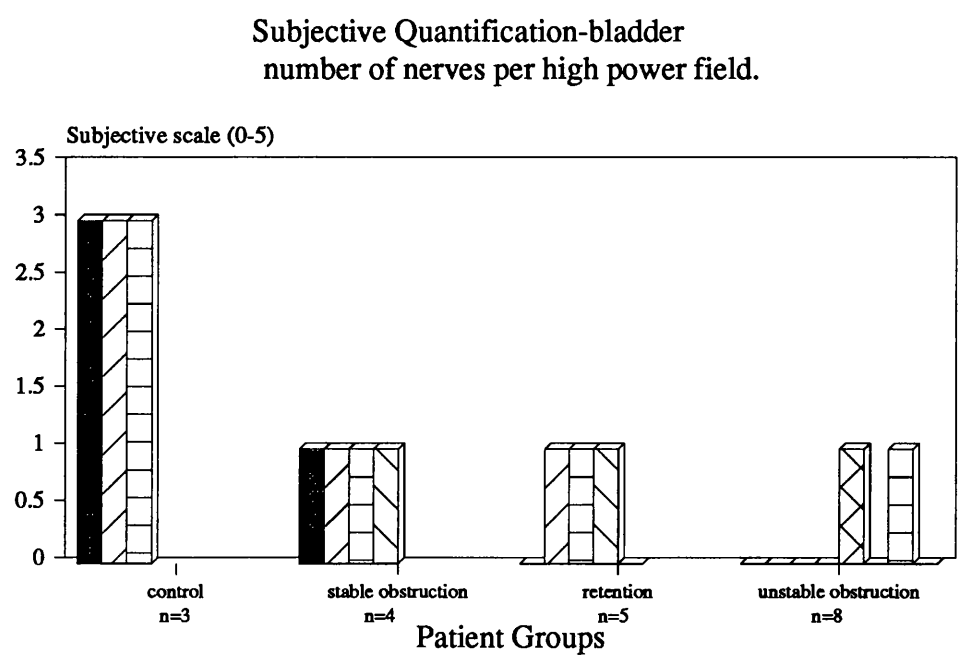
Figure 6.8

a)



CGRP- immunoreactive nerves

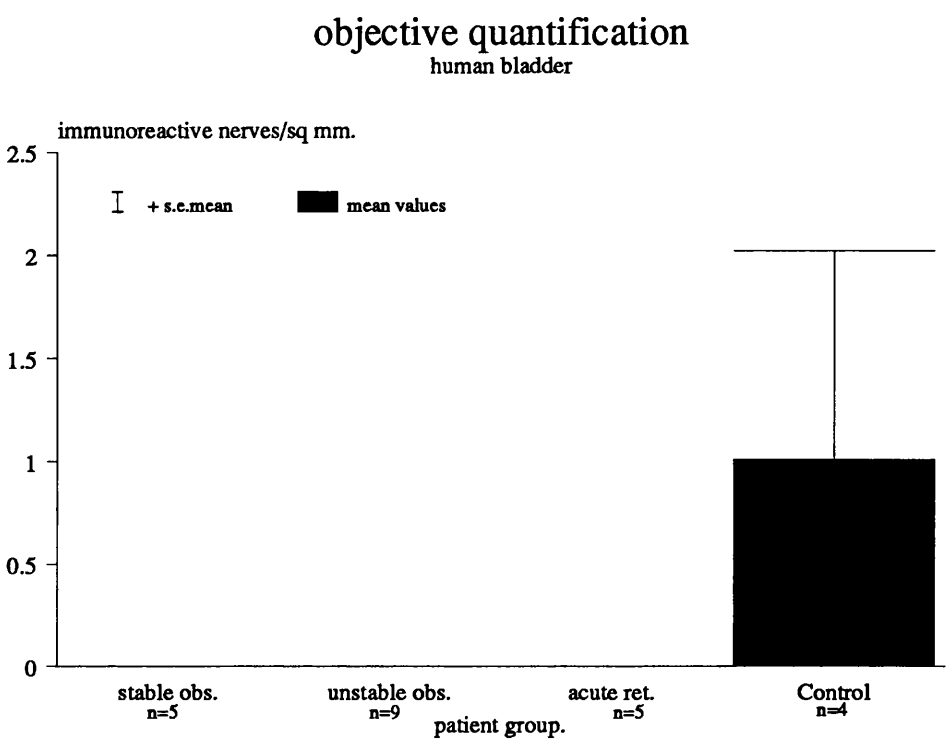
b)



CGRP- IMMUNOREACTIVE NERVES

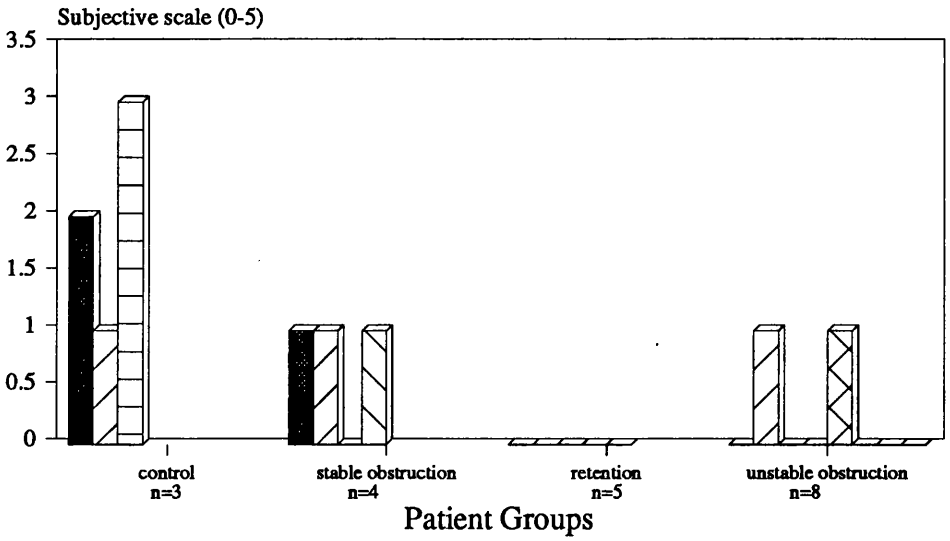
Figure 6.9

a)



SP - immunoreactive nerves

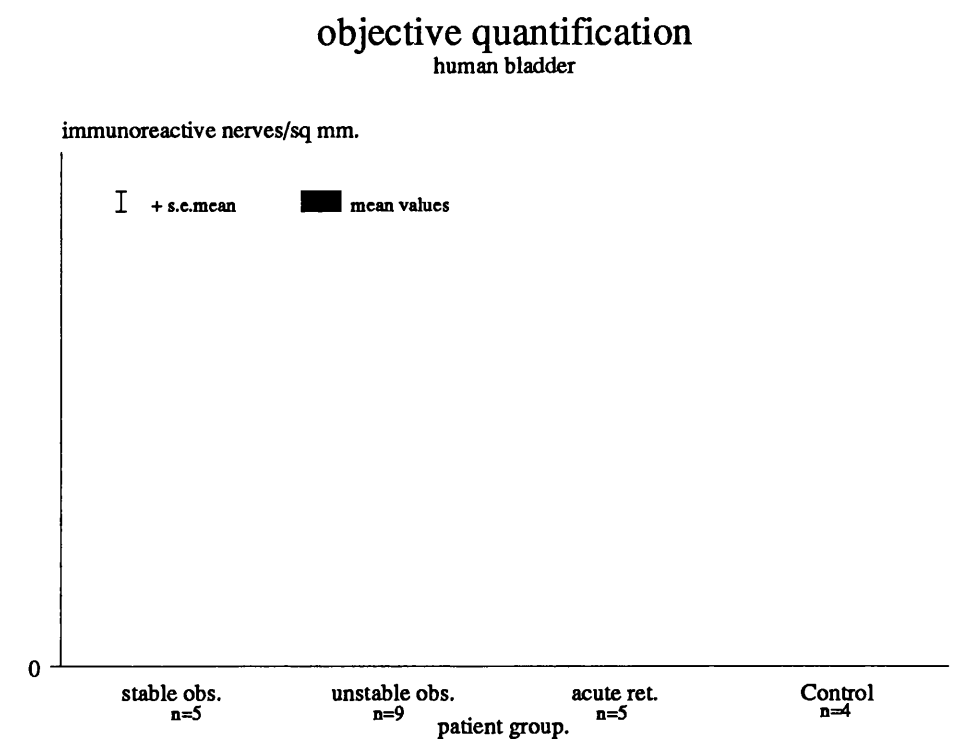
b)



SP - IMMUNOREACTIVE NERVES

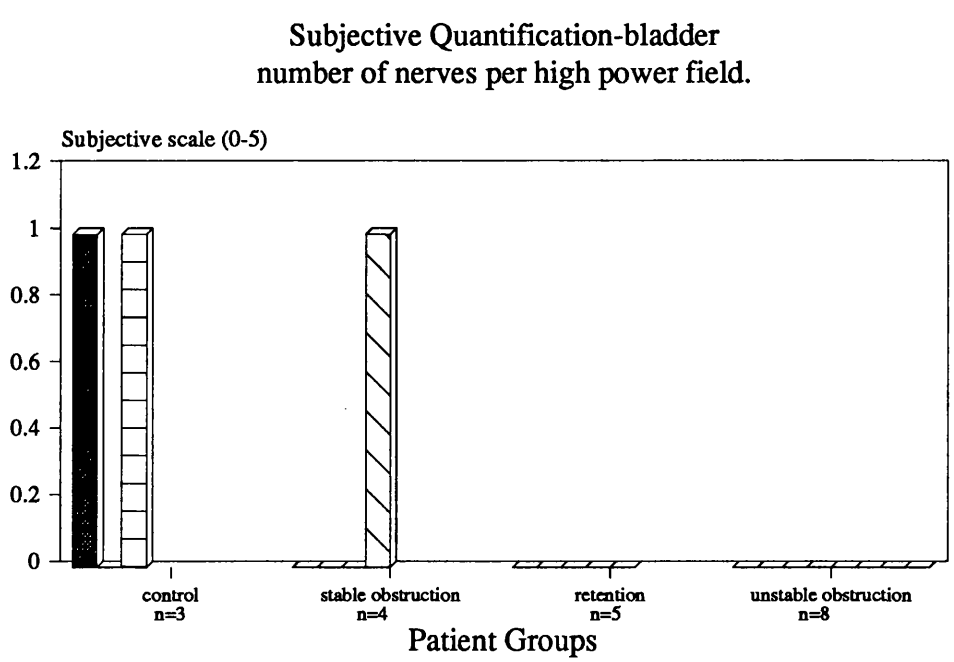
Figure 6.10

a)



SOM - immunoreactive nerves

b)



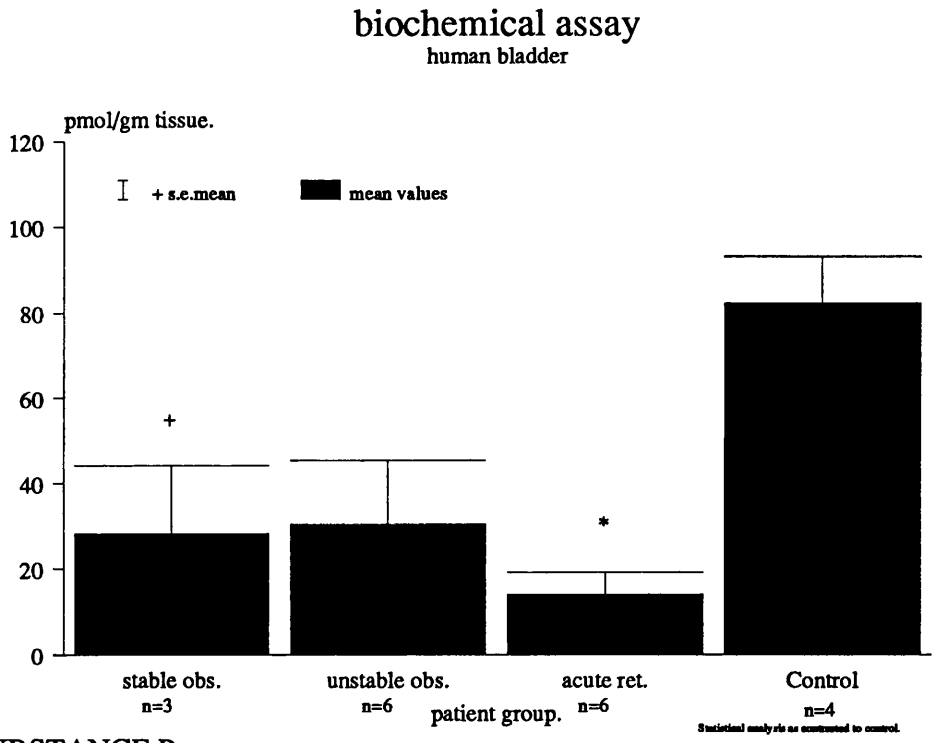
SOM - IMMUNOREACTIVE NERVES

muscle, failed to confirm this finding (see Figure 6.6a, 6.7a, 6.8a, 6.9a, 6.10a); the only significant reduction was for VIP-immunoreactivity ($p< 0.05$).

6.3.3 Biochemical Assay

The amount of bladder tissue available limited the biochemical analysis to assay of tissue of VIP, SP and CGRP (Figures 6.11-6.13). The results confirmed the presence of all three peptides within the bladder tissue. CGRP was present at a 10 to 15-fold lower concentration than either VIP or SP, whilst SP appeared to be present in a higher concentration than VIP despite the more prominent immunoreactivity for VIP seen on microscopy. The most striking change in neuropeptide concentration in the obstructed bladder body compared to controls was the reduction in SP content; obstructed stable $P<0.05$ and acute retention bladder $P<0.02$ (Figure 6.11). There was a tendency for VIP content to be lower in the obstructed bladder than the controls but this did not reach statistical significance (Figure 6.12). CGRP levels were not significantly different in any of the tissues studied (Figure 6.13).

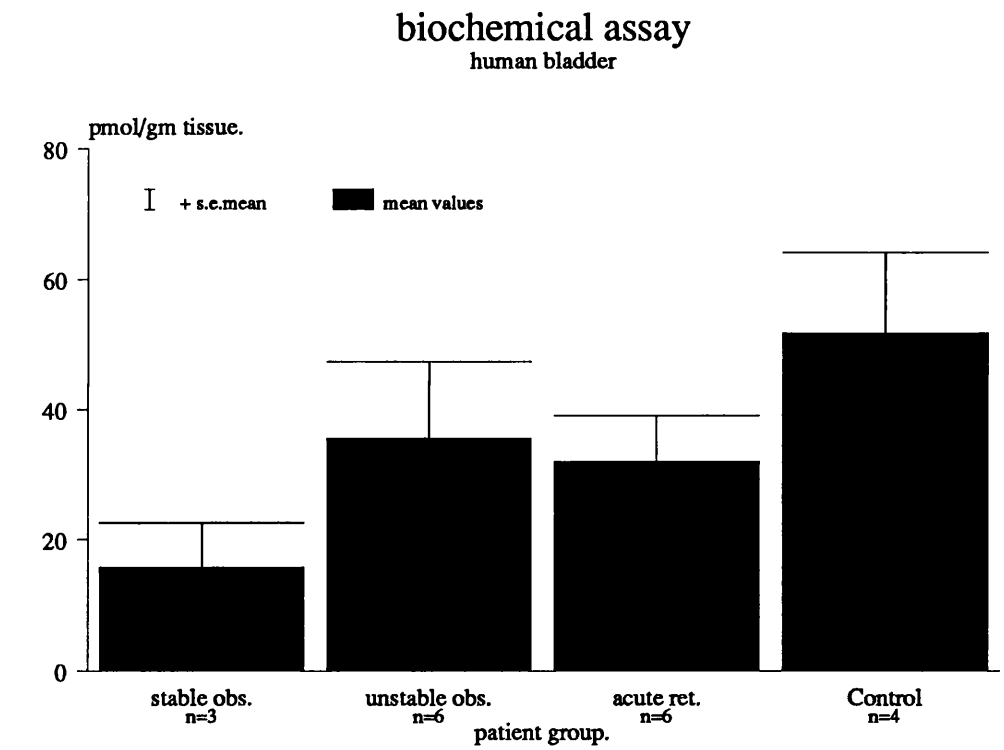
Figure 6.11



SUBSTANCE P

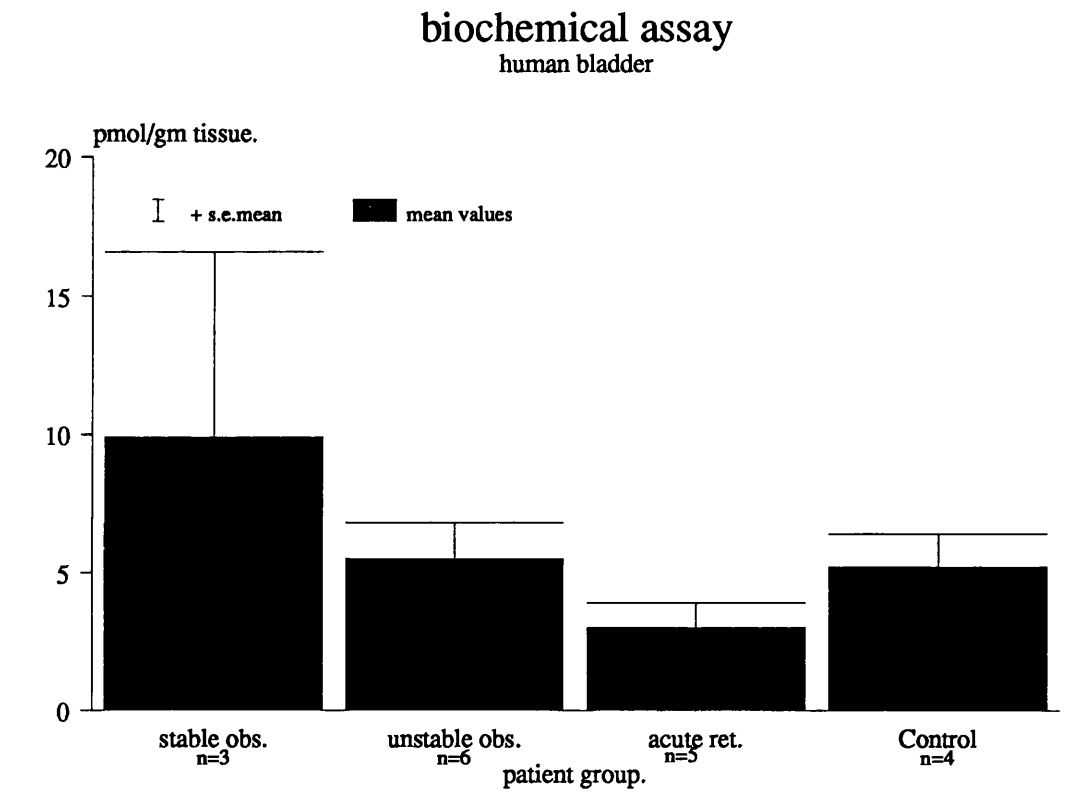
+=p<0.05: *=p<0.02 Using Mann Whitney test

Figure 6.12



VIP

Figure 6.13.



CGRP

6.4 DISCUSSION

Despite the potential importance of neuropeptide neurotransmitter mechanisms in the function of the normal and obstructed bladder, there has been little work on the localisation of neuropeptides within the normal human bladder (Gu et al. 1984, 1983b). At present, the distribution and role of peptides contained in the sensory nerves of the human bladder remains obscure. Previous studies have suggested the sensory innervation to be associated with the prominent AChE-positive nerves seen within the lamina propria of the urethra and bladder (George and Dixon 1986, Kirby 1986). In recent years it has been suggested that a number of the peptides are putative neurotransmitters within primary afferent neurons (Gibbins et al. 1987), including SP (Lembeck 1953, Nicoll et al. 1980), and CGRP, Som and VIP (Jancso et al. 1977, Sundler et al. 1985). Although previous studies have reported an abundance of CGRP-like immunoreactivity in the rat and guinea pig bladder concentrated particularly in a dense submucosal neural plexus (Yokokawa et al. 1986), the localisation of this peptide has not previously been reported within the human bladder. No previous studies have investigated the changes which occur in obstructed detrusor muscle.

In this study, subjective quantification demonstrated a reduction in the neuropeptide-like immunoreactivity of obstructed bladder. In particular, the density of immunoreactivity of nerves to VIP, CGRP and SP was markedly reduced. This finding was not reproduced by the use of objective quantitative techniques on the same slides. A possible explanation for this apparent

discrepancy is methodological. The neuropeptide-immunoreactivity of nerves within the detrusor is sufficiently sparse that a graticule-based technique would tend to produce inconsistent results owing to the large and unpredictable error introduced by the effect of graticule positioning. This is reflected by the low neural count/mm^2 muscle and the high standard error of the mean for these results. This work provides validation for the continued use of semi-quantitative assessment in studies of neuropeptide immunoreactivity.

An important factor which needs to be considered is the effect of age on bladder innervation, with a reported age-related reduction in AChE-positive nerves (Gosling et al. 1986), and sympathetic nerves (Benson et al.1979). Similar age related reduction in the enkephalinergic nerves of human prostate have been reported (Jungblat 1989). It is most unlikely that age by itself would explain the reduced density of innervation reported here, particularly as the age range of the different groups of patients studied showed considerable overlap and parallel studies of AChE staining on the same tissue did not demonstrate an age-related reduction (Chapter 5).

In order to interpret the significance of such observations it is necessary to consider the possible physiological role subserved by these neurotransmitters. It has been suggested that the putative neuropeptide neurotransmitters NPY and VIP co-exist within the same nerve fibres as the classical transmitter substances, noradrenaline and acetylcholine respectively. Since the sympathetic innervation of the normal human detrusor is known

to be sparse (Chapter 5), it is unlikely that NPY-like immuno-reactivity seen here lay solely within sympathetic nerve fibres. Some support for this interpretation is provided by the work of Mattiasson et al. (1985) who concluded, from denervation studies conducted in the rat, that there was a separate local population of NPY fibres originating from cell bodies in pelvic ganglia. The potential role of NPY in the control of detrusor muscle contraction remains unknown.

Review of the literature reveals controversy surrounding the potential pharmacological role of VIP in the bladder. Kinder and Mundy (1985a,c) reported that VIP caused a significant reduction in muscle strip basal tension and the amplitude and frequency of spontaneous contractions in normal, hyperreflexic, and unstable human detrusor and suggested that it might be acting as an inhibitory neurotransmitter. Klarskov et al. (1984a), showed that VIP produced a concentration-dependent relaxation of human detrusor. Sjogren and co-workers (1985) reported an in vitro study of the effect of VIP on human detrusor muscle strips which showed that neither acetylcholine nor electrically induced contractile responses were significantly influenced by the addition of exogenous VIP. Similarly, the intravenous infusion of VIP into male and female human volunteers (Klarskov et al. 1987c) failed to demonstrate any change in urodynamic results relating to bladder storage or voiding function. Gu et al.(1983a) reported a dramatic reduction in VIP-like immunoreactivity in the detrusor muscle of bladders exhibiting idiopathic unstable behaviour, as contrasted to normal. A much

smaller, yet significant reduction in density of VIP-like immunoreactivity, assessed using a semiquantitative technique is reported in this study, a finding not supported by the assay results.

The distribution of both NPY- and VIP-immunoreactivity noted in this study although supporting a principal role in the motor innervation of detrusor muscle, suggests that these two peptides may also participate in the regulation of blood flow. Certainly, NPY has been reported to possess a number of potent biological properties including modulation of nerve mediated muscular contraction and vasoconstriction of blood vessels (Adrian et al. 1984). It is known to be released with noradrenaline upon sympathetic nerve stimulation in man, with apparent pre- and post-junctional effects on the sympathetic control of blood vessels (Lundberg et al. 1985). VIP occurs with NPY in high concentrations elsewhere in the male genital tract and it has been suggested that both may play an important role in erectile function (Gu et al.1983b). Andersson et al. (1987) reported that stimulation of parasympathetic nerves to the bladder in the anaesthetized cat induced a marked increase in VIP output which was matched by a smaller relative increase in blood flow, thereby providing circumstantial evidence to suggest that it may not only be acting on blood vessels but may also modulate the action of other neural pathways.

CGRP and substance P have been shown to co-exist within isolated nerve fibres in the lamina propria by a number of workers (Alm et al. 1979, Yokokawa et al. 1986); with an additional population of nerves merely

containing CGRP or SP (Sundler et al 1985, Yokokawa et al. 1986). The submucosal position of these nerves is consistent with a sensory function and this is further supported by experimental studies in the rat bladder, using capsaicin, which selectively degranulates unmyelinated sensory fibres (Santicioli 1985).

Immunoreactivity to substance P and CGRP was insufficient to allow objective quantification, but semiquantitative assessment demonstrated that the density of nerves immunoreactive to these substances was substantially reduced in the obstructed bladder. Biochemical assay for substance P confirmed there was a significant reduction in the obstructed bladder which was most marked in the acute retention subgroup. There was no associated change in CGRP levels. A reduction in substance P has been demonstrated in studies using an obstructed rat bladder model (Andersson et al. 1988).

It is of note that the results obtained in this study for substance P were substantially higher than other reported values both in the human (Gu et al. 1984) and the rat bladder (Andersson et al. 1988). A possible explanation for this observation is that the bladder biopsies analyzed for substance P were obtained by the use of punch forceps and were principally composed of mucosal tissue. Since this is the main site of localisation of substance P-immunoreactive nerves, this would increase the assay result.

These findings provide the first substantive histological evidence for disturbance of the afferent innervation of the obstructed human detrusor. Recently it has been suggested that the afferent limb of the micturition

reflex may be more important in influencing bladder function than has been recognised previously. Recent study of a rat model suggests that there is an increase in local afferent spinal reflex activity in the presence of obstruction (Steers and de Groat 1988). Further studies have suggested that there is hypertrophy of nerves innervating the bladder (Steers et al. 1989). A tempting explanation for this latter observation is that this hypertrophy represents a secondary phenomenon in response to the local detrusor denervation.

At present the observation that a decrease in intravesical substance P levels is directly associated with increased bladder activity cannot be satisfactorily explained. The application of capsaicin to the animal bladder produces a profound reduction in both substance P and CGRP content which results in the opposite effect, an increased bladder capacity (Maggi et al. 1986) and even urinary retention (Sharkey et al. 1983). The situation is further complicated by the reported action of substance P and the related group of neurokinins in producing contractile effects in the mammalian bladder (Maggi and Meli 1986, Dion et al. 1988, Erspamer et al. 1981). However, Kalbfleisch and Daniel (1987) in studies on human bladder challenge the suggestion that these contractile motor effects represented a true physiological response, since selective substance P tachyphylaxis and a substance P inhibitor failed to reduce the NANC response to field stimulation.

It is clear from this investigation that a number of putative

neuropeptide transmitters occur in the human detrusor and that their concentration is reduced in the presence of obstruction. Although a fuller interpretation of these findings remains highly speculative and awaits further functional and ultrastructural study, the aetiology of obstructive detrusor instability is clearly related to a more widespread disruption of the normal structure and function of the human detrusor than has been previously recognised. Further study of the localisation of neuropeptides and their pharmacological action within the normal and obstructed human detrusor could possibly provide new therapeutic options for the control of functional disorders of the bladder.

CHAPTER 7

ANATOMY AND INNERVATION OF THE PROSTATE GLAND

7.1 INTRODUCTION

Although its true function is unknown, it is likely that the human prostate gland plays a vital role in reproductive physiology. Persistent debate in the literature over both structure and terminology have limited our understanding of prostatic anatomy until recent years. Morgagni (1769) was amongst the first to recognise the predilection of prostatic disease for older men and both benign and malignant prostatic enlargement are important clinical problems from the sixth decade of life onwards.

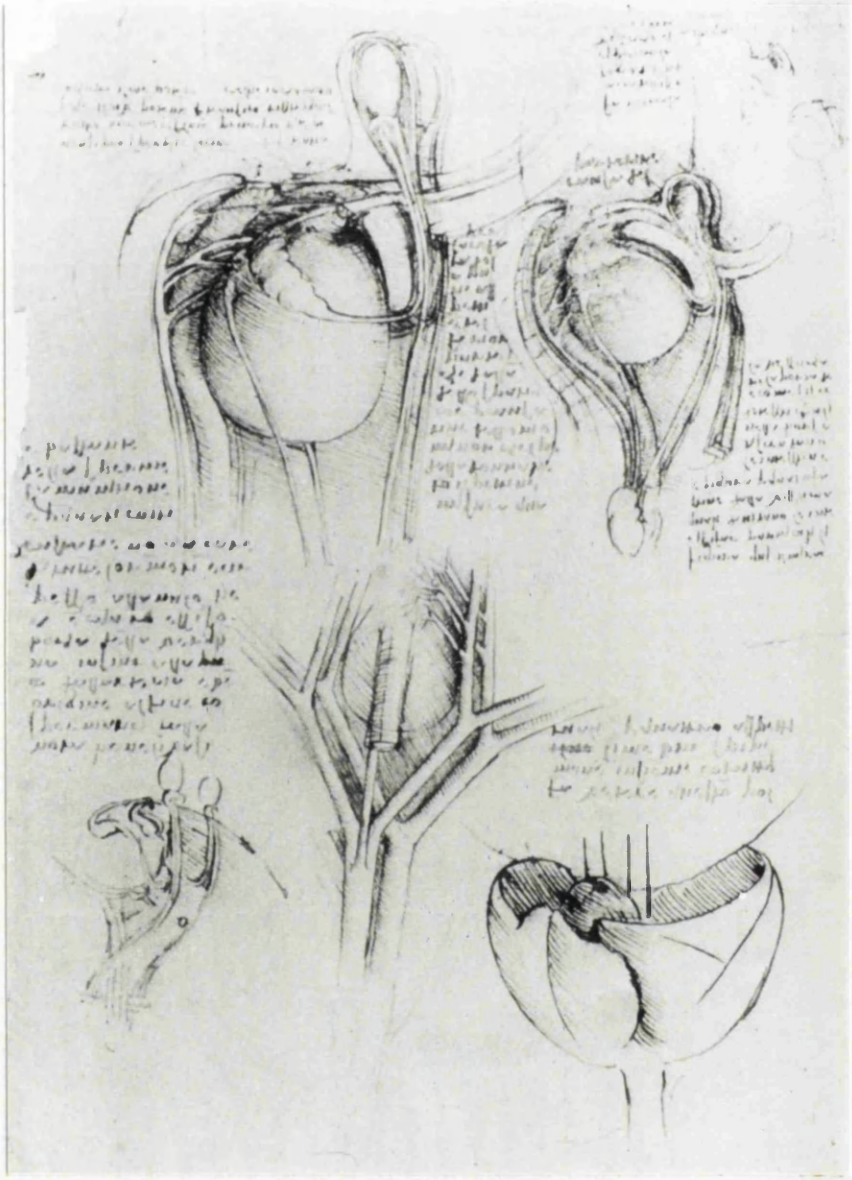
7.2 ANATOMY

The prostate gland has been forgotten and rediscovered a number of times during recorded medical history. The term *prostate* is attributed to Herophilus circa 300 BC (Galen) and was applied by him to the structures now called seminal vesicles. Accurate anatomical studies by Nicolo Massa of Padua (circa 1550) and Vesalius depicting the prostate gland first appeared in the 16th century (Shelley 1969), but it was noticeably absent from the anatomical drawings of Leonardo da Vinci (O'Malley 1952, Figure 7.1).

Lowsley (1912) was the first to publish a detailed description of the anatomy of the prostate; he described five prostatic lobes and this system of anatomical subdivision still remains in common use today. This work is based on a study of foetal prostates and it is therefore difficult to justify the extrapolation of such results to the interpretation of adult anatomy; for

Figure 7.1.

Diagram from the anatomical works of Leonardo da Vinci (Courtesy of the Royal Collection, Windsor), demonstrating the human bladder and lower urinary tract but with no evidence of a recognisable prostate gland.



example, the anterior lobe described by Lowsley atrophies before birth. Franks (1954) modified Lowsley's formulation, introducing the concept of central and peripheral prostate with benign hyperplasia arising in the central zone and carcinomatous disease in the peripheral zone.

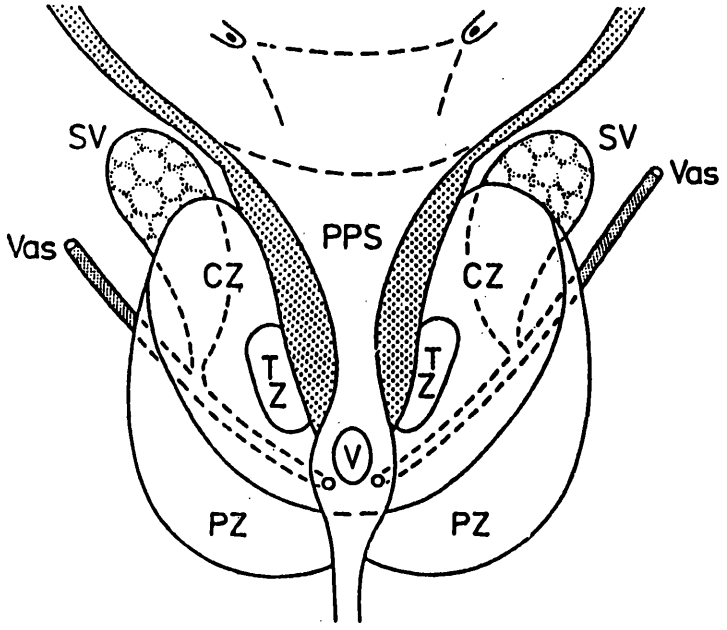
McNeal (1972) has reported the most detailed study of prostatic anatomy to date. He documented anatomical heterogeneity within the prostate and expanded the concept of central and peripheral zones, to include a sub-division of the central zone encompassing peri-urethral glands, *the transitional zone*; which he suggested as the site of origin of benign prostatic hyperplasia (McNeal 1978 a,b, see Figure 7.2). He described a cylindrical sphincter of smooth muscle surrounding the urethra from the upper end of the verumontanum to the bladder neck, *the pre-prostatic sphincter*. Gosling, Dixon et al. (1983) confirmed the presence of a circularly disposed smooth muscle collar around the pre-prostatic urethra which was continuous proximally with the bladder neck and merged distally with the capsule of the prostate gland and contributed a fibromuscular covering around the prostate gland.

Tisell (1975), in a study based on the dissection of gross specimens, reported cleavage planes which separated three regions with microscopically different appearances, which he named middle, lateral and posterior lobes. These lobes are different to those reported by Lowsley but can be equated with the work of McNeal, the middle lobe corresponding to the central zone and the posterior or lateral lobes to the peripheral zone (McNeal 1980).

The prostatic urethra is continuous above with the pre-prostatic urethra and emerges from the prostate slightly anterior to the apex of the gland (Figure 7.3). Throughout most of its length there is a mid-line posterior ridge the urethral crest, which is most prominent at its midpoint, the verumontanum which is situated at the proximal end of the distal urethral sphincter mechanism (Gosling, Dixon et al. 1983). The prostatic urethra takes its name from the surrounding prostate gland which contains a prominent muscular component which is particularly marked in benign prostatic hyperplasia (Bartsch 1979). Slender, smooth muscle bundles occur in the proximal part of the urethral crest continuous above with the superficial trigone and below with the prostatic urethra where they merge with the muscle coat of ejaculatory ducts. Distally the prostatic urethra possesses a thin, smooth muscle coat comprising circular and longitudinal muscle and merging with the prostatic musculature, enhanced by an outer circular coat of striated muscle continuous with the distal sphincter mechanism (Gosling and Dixon 1975, Benoit et al. 1988).

Obstruction arising in association with benign prostatic hyperplasia is an important clinical problem, which has been traditionally attributed solely to the mechanical effect of prostatic enlargement. Recent work has suggested that nearly 50% of prostatic outflow obstruction is mediated via neural pathways acting on the smooth muscle of bladder neck, pre-prostatic and prostatic smooth muscle (Furuya et al. 1982). This has renewed interest in a therapeutic role for α adrenoceptor blockade in the treatment of benign

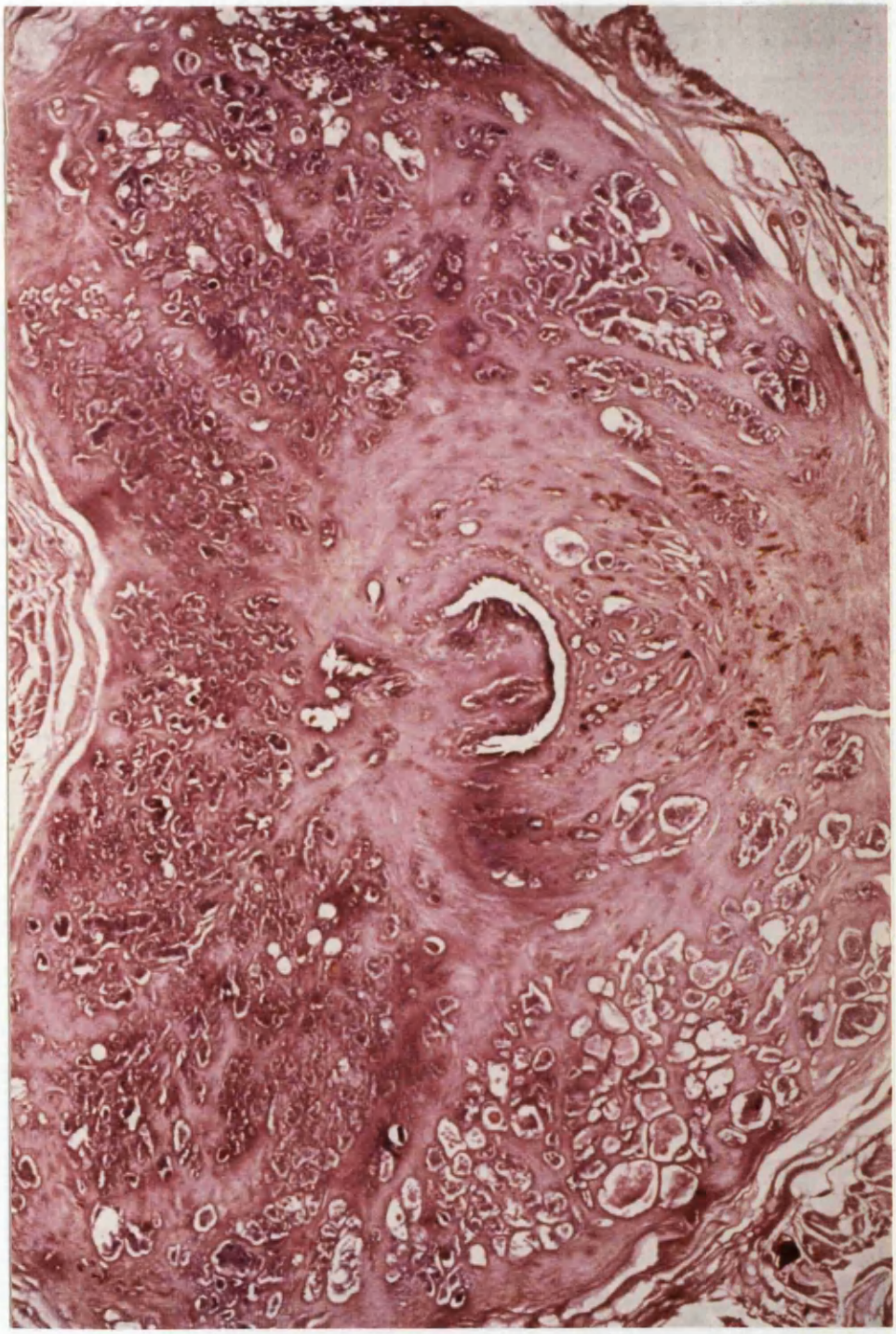
Figure 7.2.



Schematic diagram of adult prostate showing peripheral zone (PZ), central zone (CZ) and transitional zone (TZ) at apex of pre-prostatic Sphincter (PPS). Seminal vesicles (SV) and vasa deferentia fuse to form ejaculatory ducts to open alongside verumontanum (V).

Figure 7.3.

Transverse section through the prostate gland showing the centrally situated prostatic urethra and the urethral crest (Haematoxylin and Eosin, courtesy of Professor J. Gosling).



prostatic hyperplasia, a subject which is considered in some detail in this thesis (Chapters 9 and 10). A detailed review of literature on the innervation and pharmacological response to the prostate gland and prostatic urethra is therefore included in the next section.

7.3 INNERVATION

Early investigations dealt almost exclusively with the extrinsic innervation of the gland (Langley and Anderson 1894, 1895, 1896). Later studies turned to the intrinsic innervation of the prostate and numerous authors sought to describe the various nerve types within the prostate (Casas 1958), but were limited by lack of suitable staining techniques. It was widely held that some of these nerves were sensory (Seto 1954), whilst others were motor to blood vessels and stroma (Mori 1955). The advent of histochemical techniques for the demonstration of catecholamines (Corrodi and Jonsson 1967) and acetylcholinesterases (Koelle 1951 and Gomori 1952), made possible the localisation of these nerve types and subsequent work in the last two decades has introduced a separate group of non-adrenergic non-cholinergic sensorimotor nerves. It is now recognised that the human prostate gland is innervated by short adrenergic sympathetic nerves whose cell bodies lie in the pelvic plexuses (Baumgarten et al. 1968), cholinergic parasympathetic nerves (Duzendorfer et al. 1976) and peptidergic sensorimotor nerves (Larsson et al. 1977, Alm et al. 1977, Gu et al. 1983b).

Despite the clinical importance of the prostate gland, few comprehensive studies of the innervation and ultrastructure of the human prostate and

associated prostatic urethra have been reported. It is of interest that the human prostate gland receives a considerably less dense innervation than most laboratory animals (Baumgarten et al. 1968, Shirai et al. 1973). This highlights the importance of exercising caution in extrapolating results from animal studies to man.

It has been suggested that although microscopically there is dual innervation to all parts of the prostate: the glandular acini are principally supplied with secretomotor cholinergic parasympathetic nerves and the predominant motor control of prostatic muscle is via sympathetic adrenergic neurons (Gosling 1983). This suggestion has however been challenged by Vaalasti and Hervonen (1980a), who dispute that there is a significant sympathetic or parasympathetic nerve supply to acinar epithelium. As mentioned above there is a third group of nerves containing putative peptide neurotransmitters. Although they have been reported to innervate the prostate both in man (Gu et al. 1983b) and the cat (Alm et al. 1980), detailed characterisation of their distribution in the human prostate has not yet been reported. The precise role of these nerves is obscure at present although, based on other observations, it is likely that they have an important role in controlling vascular tone. In addition, they may be important in neuromodulation, since VIP and NPY are known to coexist with classical transmitters within cholinergic and adrenergic nerves respectively.

The motor control of the pre-prostatic and prostatic urethra appears to be similar to that of the adjacent prostate and is mediated predominantly

via the sympathetic nervous system (Andersson et al. 1983). It is however difficult to equate this observation with ultrastructural studies. These demonstrate that the human urethra receives a sparse supply of adrenergic nerves, a rich supply of AChE-positive neurons (Ek et al.1977) and also contains peptide neurons such as VIP (Alm et al. 1980) and NPY. The adrenergic nerve supply and presumably other nerves enter the prostate alongside the ejaculatory ducts and then ramify superiorly towards the bladder neck and inferiorly to innervate the infra-montanal prostatic urethra (Benoit 1988).

Autonomic ganglia staining for AChE (Gosling 1983, Kluck 1980) and noradrenaline (Gosling and Thompson 1977) have been described in the prostatic capsule, and AChE-positive ganglia have been described within the prostatic stroma (Dunzendorfer et al. 1976). More recently, VIP-like immunoreactive ganglia have been described in the human prostate (Gu et al. 1983) and in the cat (Alm et al. 1980) and human urethra (Crowe et al. 1988). These observations suggest that many of the immunoreactive nerves arise locally and raises the possibility of a significant local neural interaction.

Two studies have reported that there is a reduced innervation of adenomatous as contrasted to normal prostate, affecting both the cholinergic and adrenergic components (Dunzendorfer et al. 1976). This could result from either a dilution of neural structures by the marked increase in muscular and to a lesser extent glandular components within the hyperplastic gland

(Bartsch 1979), or could represent a true reduction in innervation. The latter suggestion is supported by in vitro isometric studies which have demonstrated an exaggerated response to adrenergic agonists of hyperplastic as compared to normal prostatic tissue, suggestive of a post-junctional supersensitivity (Kitada 1987). This supersensitivity may contribute to the clinical picture of prostate obstruction.

Learmonth (1931) reported that stimulation of the pre-sacral nerve in man contracted the prostatic musculature. Since then, in vivo urethral pressure profile studies (Donker et al. 1972) and in vitro isometric prostatic strip studies (Caine 1986b) have convincingly confirmed the important influence of the sympathetic nerve system on the bladder outflow tract, thereby raising the potential for pharmacological treatment using α blockade. Initial experience with a non-selective α antagonist, phenoxybenzamine, has been disappointing because of the unacceptably high incidence of side effects. These have been attributed to a blockade of presynaptic α_2 receptors thereby interfering with the normal negative feedback control of the release of noradrenaline. The current model suggested to explain the distribution and role of α receptors in the sympathetic control of the prostate is illustrated diagrammatically in Figure 7.4.

With the recognition of the importance of the α_1 receptor in mediating sympathetic action in the normal and adenomatous prostate, attention has turned to the therapeutic use of selective α_1 antagonists such as prazosin (Shapiro et al. 1981), with the therapeutic goal of producing a more potent

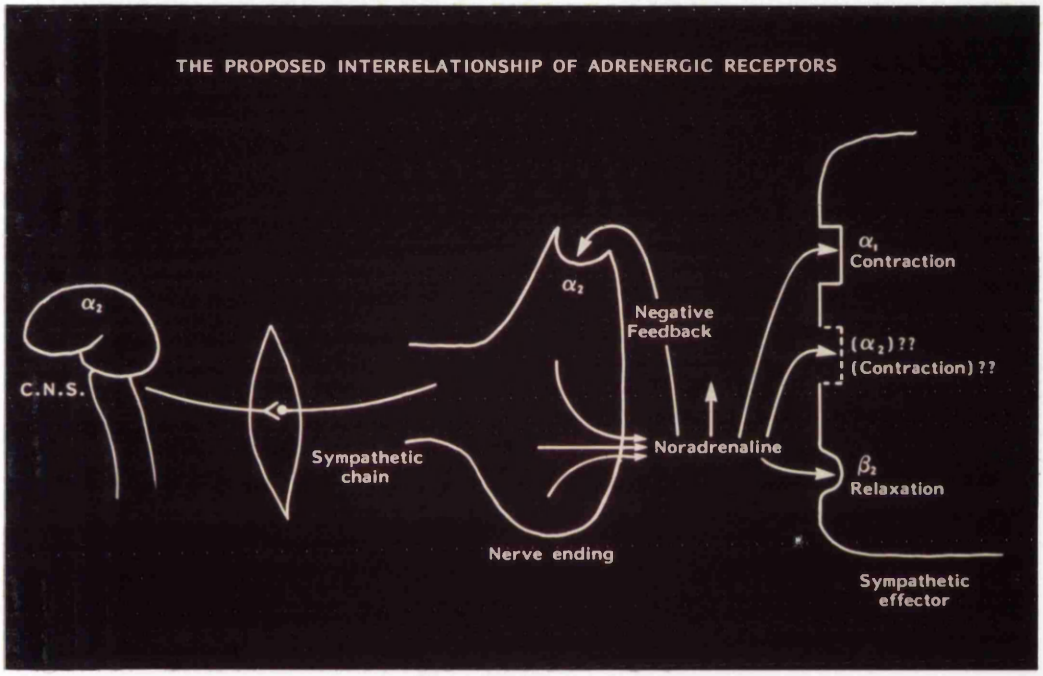
action combined with less unwanted side effects. Although numerous in vitro isometric studies have been reported in the literature, only one study has also investigated normal prostatic tissue (Hedlund 1985).

Ligand binding techniques have only recently been applied to this area with two groups having reported on the characterisation of human prostatic α_1 and α_2 adrenoceptors. These studies have raised an as yet unexplained paradox. Hedlund et al. (1985) demonstrated an excess of α_2 over α_1 receptors in adenomatous prostate despite a functional predominance of α_1 receptors; Lepor and Shapiro (1984) and Shapiro and Lepor (1986) suggested equivalent densities for both groups of α receptors. In support of this latter observation, it has been suggested that α_2 receptors may have an important role in mediating the contraction of prostatic smooth muscle (Shapiro et al. 1987).

Lepor and Kuhar (1984) characterised the muscarinic cholinoceptors in human prostatic tissue using radioligand receptor binding and slide mounted autoradiographic techniques and reported high affinity muscarinic cholinoceptors localised to prostatic glandular tissue, suggesting that this is the main site of action of the parasympathetic innervation. No specific localisation studies have so far been reported for prostatic adrenoceptors.

Similar controversy surrounds the subject of urethral innervation, with little available information as to the functional importance of cholinergic and neuropeptide containing neurons. Investigations both in vivo and in vitro have suggested that the smooth muscle of the human urethra contains both adrenoceptors and muscarinic receptors (Andersson and Sjogren 1982). Whilst

Figure 7.4.


THE PROPOSED INTERRELATIONSHIP OF ADRENERGIC RECEPTORS
α_2
C.N.S.
α_2
Negative Feedback
Sympathetic chain
Noradrenaline
Nerve ending
α_1 Contraction
(α_2) ??
(Contraction) ??
β_2 Relaxation
Sympathetic effector

adrenergic stimulation has been demonstrated to produce a contractile response, neither muscarinic receptor agonists nor antagonists have any significant effect on intra-urethral pressure (Ek et al. 1978).

It is likely that there is an interaction between cholinergic and adrenergic nerves in the prostate, urethra and bladder neck (Nergardh 1975, Mutoh et al. 1987, Ek et al. 1977). Mattiasson et al. (1984) in a study conducted on the isolated human urethra showed that stimulation of muscarinic receptors caused a significant decrease of the electrically induced release of ^{3}H noradrenaline, suggesting that activity in cholinergic parasympathetic nerves could influence sympathetically controlled noradrenaline dependent urethral tone.

Some evidence has accumulated to support NANC neurotransmission in the control of urethral function. Andersson et al. (1983) reported that electrically induced TTX-sensitive relaxant responses both in human inframontanal urethra pre-contracted with noradrenaline and in corresponding rabbit experiments supported a NANC sensorimotor mechanism of action. Further experiments reported by Mattiasson et al. (1985) demonstrated an electrically induced contraction in both rabbit and human urethra which was suggestive of NANC neurotransmission, but which could not be blocked in all cases with TTX. Non-adrenergic non-cholinergic neurotransmitters may be contained within interneurons between the cholinergic and adrenergic systems, playing a role as neuromodulators, functioning as cotransmitters, or producing a local trophic effect.

It is evident from this review that there are a number of questions as to the role of the adrenergic and cholinergic innervation of the prostate/urethral complex remain unanswered. No investigators have so far demonstrated the differential ultrastructural localisation of cholinoceptors and adrenoceptors within the human prostate. In addition, the detailed localisation of specific neuropeptides within these tissues has not been reported. Such a study is an essential prerequisite to further understanding of the potential role of the non-adrenergic non-cholinergic innervation of the prostate. Experimental studies carried out to clarify these points are presented in chapters 8, 9 and 10.

CHAPTER 8

THE INNERVATION OF THE HUMAN PROSTATE GLAND - THE CHANGES ASSOCIATED WITH BENIGN ENLARGEMENT.

8.1 INTRODUCTION

The prostate gland plays an important role in reproductive physiology and commonly undergoes benign hyperplastic enlargement in the elderly male. This occurs with a reported prevalence of 37-40% during the fifth decade rising to 75-84% during the eighth decade of life (Moore 1935, Franks 1954). Indeed, it has been suggested that the chance of a 40-year old man having a prostatectomy during his lifetime is 29% (Glynn et al. 1985). Secondary detrusor instability occurs in 49-80% of these obstructed patients (Abrams 1985).

It is now established that the prostate gland receives innervation from nerves containing noradrenaline and acetylcholine (Dunzendorfer et al. 1976, Baumgarten et al. 1968) and a number of sensorimotor nerves containing, vasoactive intestinal polypeptide (VIP), met- and leu-enkephalin (m-Enk) and (l-Enk) and neuropeptide Y (NPY) (Larsson et al. 1977, Vaalasti et al. 1980c; Gu et al. 1983b; Adrian et al. 1984). 5-hydroxytryptamine (5-HT)-containing glandular neuroendocrine cells have also been described in the human prostate (Sant d'Agnese et al. 1985a,b). The important role played by the sympathetic nervous system in the motor control of prostatic musculature has been more clearly delineated in recent years, with reports from in vivo (Furuya et al. 1982, Donker et al. 1972), in vitro and clinical studies (Caine

et al. 1975, 1986a,b) and forms the basis of the therapeutic use of α blockade in the medical management of benign prostatic hyperplasia. Although initial in vitro studies of prostatic capsule demonstrated it to have a significant cholinergic innervation (Vaalasti and Hervonen 1980a) and to mount a contractile response to cholinergic stimulation (Caine et al. 1975), subsequent reports have failed to provide evidence that the parasympathetic system plays a significant motor role within the prostate (Bruschini et al. 1978, Hedlund et al. 1985). A decrease in the density of adrenergic and acetylcholinesterase (AChE)-positive nerves has been reported in hyperplastic prostatic tissue in man (Dunzendorfer et al. 1976, Vaalasti & Hervonen, 1980a).

Despite the undoubted clinical importance of the prostate, it is surprising to note the limited knowledge currently available on the ultrastructure and in particular the innervation of the normal and hyperplastic gland. Although it is likely that the changes in innervation follow the ultrastructural abnormality, the possibility remains that whether primary or secondary they are likely to influence the clinical presentation of the condition.

No previous workers have comprehensively studied the distribution of classical and putative neurotransmitters with reference to the degree of regional prostatic heterogeneity, the altered innervation of the hyperplastic prostate and the correlation of such observations with the clinical and urodynamic features of prostatic obstruction. In this study, the distribution

of the neuropeptides, VIP, m-Enk, l-Enk, NPY, substance P(SP) and calcitonin gene related peptide (CGRP), the amine 5-HT and the enzymes acetylcholinesterase (AChE) and dopamine-β-hydroxylase (DBH) (indicative but not specific for acetylcholine- and noradrenaline-containing nerves respectively have been investigated in several regions of the prostate gland from patients with and without bladder outlet obstruction.

8.2 MATERIALS and METHODS

8.2.1 Patients and Tissue Collection

In the study group, specimens of prostatic tissue were obtained from patients undergoing routine surgery for the relief of bladder outlet obstruction consequent upon benign prostatic hyperplasia. Prostatic tissues were obtained either by endoscopic resection, or during open prostatectomy. All patients who had endoscopic resections had all tissue obviously damaged by diathermy excised prior to processing. Potential artefact could be introduced by the use of electrocautery during endoscopic resection. Personal observations have failed to support the possibility that significant histological and histochemical changes follow. This has been confirmed by a recent study comparing the ligand binding properties of tissue obtained at open surgery and following electrocautery resection (Lepor et al. 1988). Tissues were collected in the operating theatre and fixed to cork using "Tissuetek" and either frozen in isopentane (2-methylbutane) previously cooled in liquid nitrogen to -160°C or fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for two hours and washed thoroughly with 7% sucrose

(ANALAR-Sigma) in PBS containing 0.01% sodium azide (BDH) and stored for at least 24 hours at 4°C. This tissue was then submitted to histochemistry or immunohistochemistry respectively, as described previously (see Chapters 5 and 6).

Tissue was obtained from 32 patients comprising the following groups:- stable obstructed, mean age 67.13 ± 3.14yrs (n=8), unstable obstructed, mean age 67.08 ± 2.08yrs (n=13), acute retention of urine mean age 69.55±2.08yrs (n=11). This subdivision was based on a full urodynamic assessment carried out on all patients pre-operatively using videocystometry (Bates 1970, see Chapter 5).

In the control group, tissue samples were obtained from 5 patients undergoing radical cystoprostatectomy for carcinoma of the bladder, none of whom had received prior radiotherapy (mean age 66.2 ± 5.98 years). These patients were therefore similarly age-matched to those in the main study group and had no clinical signs or histological evidence to suggest bladder outlet obstruction. It must be emphasised that it is difficult to obtain age-matched non-hyperplastic prostate since this condition has such a high prevalence in this age group. The alternative possibility using comparison to a young population should be avoided because it does not take into account the potential decrease in innervation which may occur with advancing age, such as reported previously for the bladder (Gosling 1987).

In order to investigate heterogeneity in the innervation of the prostate, several regions of the gland were sampled wherever possible, namely:-

anterior capsule, peripheral zone and central zone which was further subdivided into three areas; near the bladder neck (proximal), near the verumontanum (distal) and midway between these two sites.

8.2.2 HISTOLOGICAL TECHNIQUES

Light microscopy

Cryostat sections of all the prostatic samples were stained with haematoxylic and eosin to allow a morphological assessment to be carried out.

Histochemistry

Cytostat sections of the different regions of the prostate were fixed in 10% formalin and 1% calcium chloride. AChE was localised according to the method of Gomori (1952, see Chapter 5). The tissues were viewed using light microscopy and areas were photographed on Ilford FP4 film.

Immunohistochemistry

An indirect fluorescence technique (Coons et al. 1955) was used to investigate the presence of immunoreactive nerves to NPY, VIP (RIA (UK) Ltd), SOM (DAKOPATTS), m-Enk (Peninsula), l-Enk (Peninsula), CGRP (CRB (UK) Ltd), 5-HT (Immuno Nuclear Corp) and DBH (Eugene Tech International Inc), in cryostat sections (10 μm) of the prostate (see chapter 6 for further details of methods).

Quantification

A semi-quantitative method (assessed blind) was used to establish the density of nerves containing the putative peptide neurotransmitters, 5-HT

and DBH.

An objective quantitative technique, was used for the analysis of AChE-positive staining (also read blinded), using techniques previously described (Gosling et al. 1986). A minimum of ten high power fields were examined from each specimen; all results were then standardised by reference to a separate quantification of the relative proportions of stromal and glandular components in each specimen. The effects of artefact attributable to stromal tissue hyperplasia were thereby eliminated. Further potential errors could still arise through hypertrophy of individual muscle cells within the stromal compartment; however, previous studies using stereological analysis have failed to demonstrate any significant differences in stromal volume density when contrasting central and peripheral hyperplastic prostate and central normal prostate (Bartsch et al. 1979).

8.3 RESULTS

8.3.1 Histology

The haematoxylin and eosin stained sections of the different regions of the prostate showed that the anterior capsule is almost entirely composed of smooth muscle fibres and fibroelastic connective tissue. The peripheral zones contained predominantly smooth muscle fibres and connective tissue with glandular alveoli and ducts. The different regions within the central prostate contained numerous mature fibroblasts in loose transparent connective tissue, numerous alveoli and ducts, but fewer smooth muscle fibres when compared with the anterior capsule and peripheral prostate. It must however be emphasised that these comments relate to the general appearances with considerable heterogeneity in tissue composition being evident in each region (Figure 8.1). In all patients undergoing surgery for the relief of bladder outflow obstruction, there was as might be expected, a more pronounced glandular hyperplasia than that seen in control patients.

Intrinsic ganglia were found in all areas of the prostate studied. In each region 1-8 ganglia, each containing 3-59 nerve cell bodies (diameter 25-40 μm) were found (Figures 8.2, 8.3, 8.4, 8.5, 8.6, 8.7). The greatest number of nerve cell bodies were found in the proximal and distal regions of the central prostate with the least in the peripheral prostate.

Figure 8.1

Section of human prostate stained using the Gomori technique demonstrating acetylcholinesterase-positive nerves, shown in brown. Note the heterogeneity of the innervation (magnification x60).

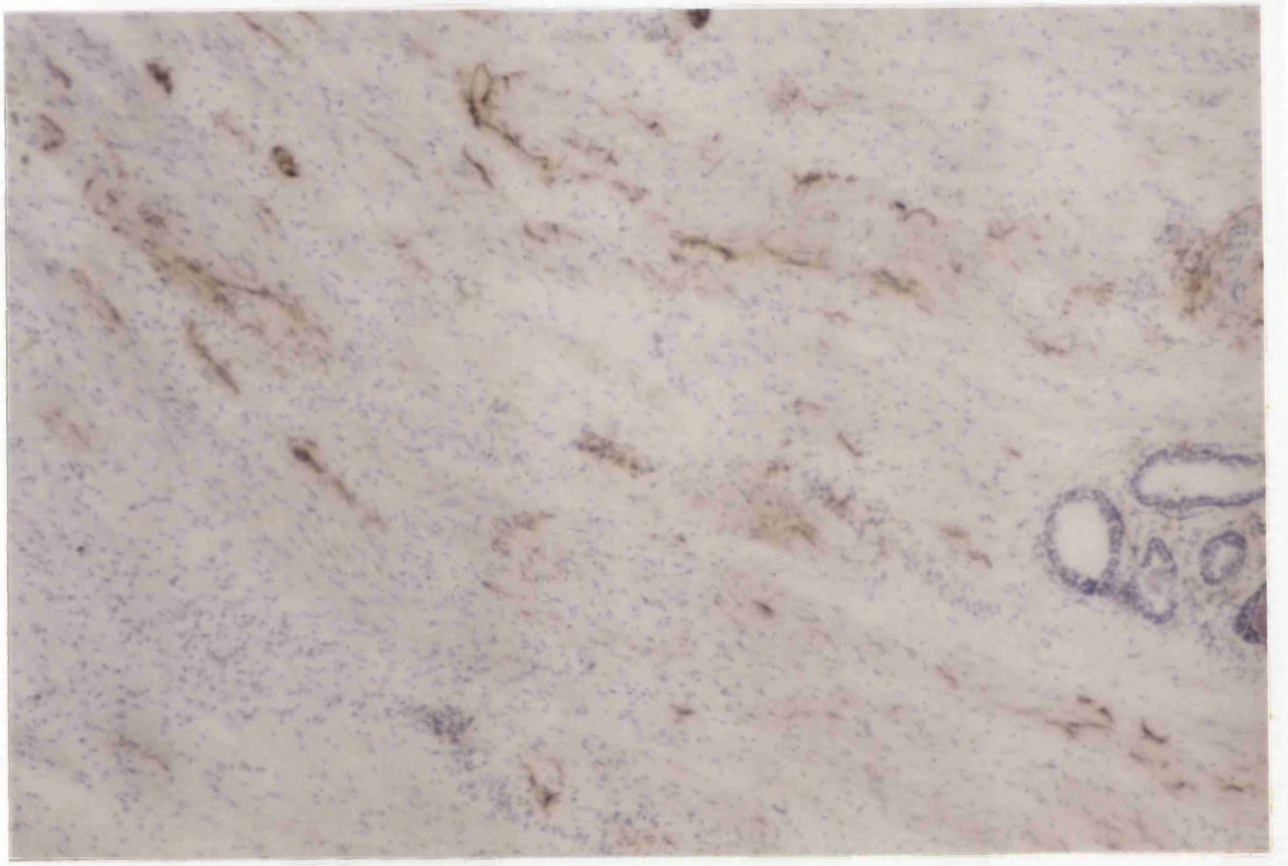
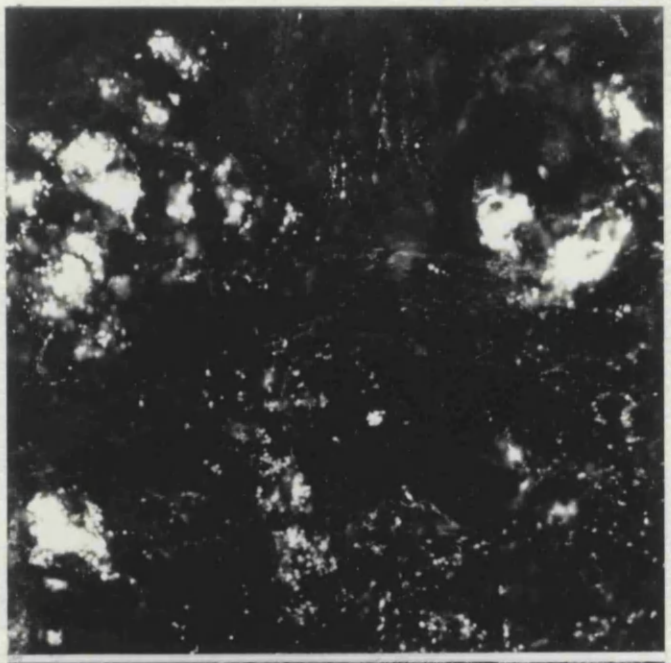


Figure 8.2

m-Enk-immunoreactivity in sections of human prostate (scale 1cm=30μm).
a) A ganglion containing m-Enk immunoreactive nerve cell bodies and nerve cell fibres in the distal central prostate.
b) m-Enk-immunoreactive nerves are seen in the periacinar stromal tissue in the distal central prostate.

a)



b)

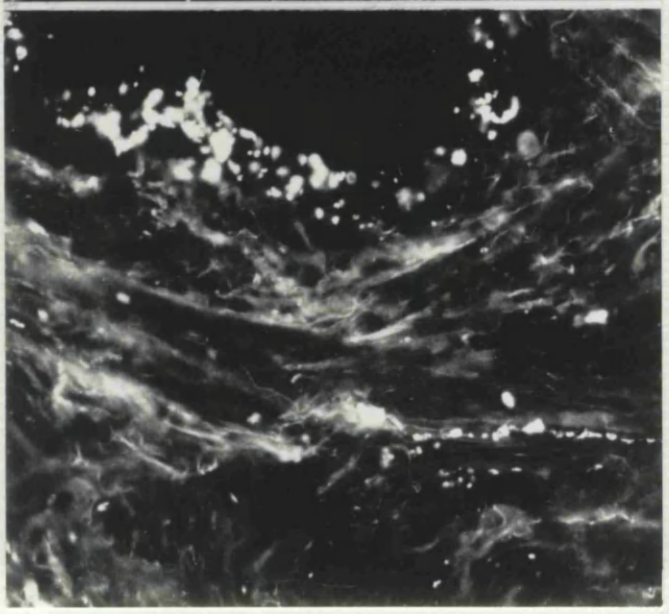
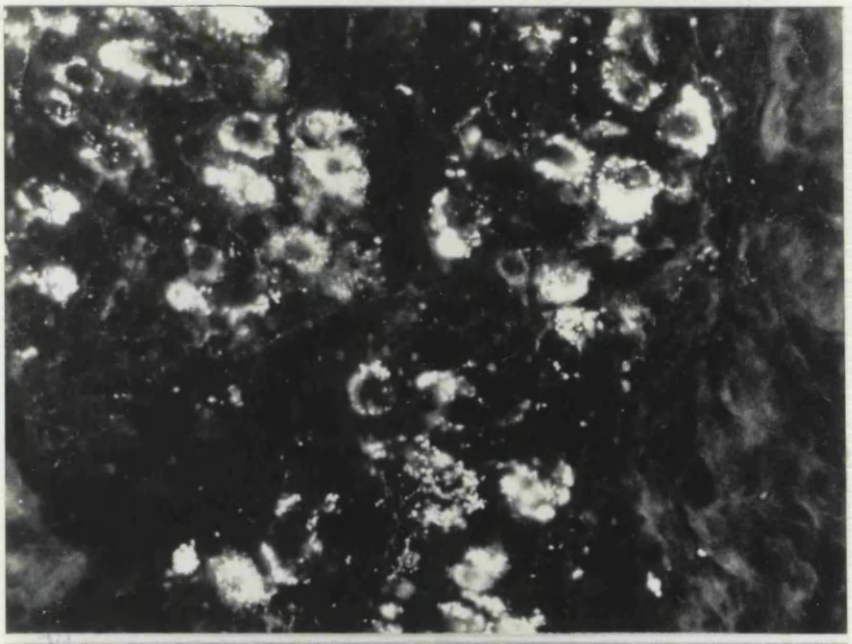


Figure 8.3

l-Enk immunoreactivitv in sections of human prostate (scale 1cm=30um).
a) A ganglion containing l-Enk immunoreactive nerve cell bodies and a few associated immunoreactive nerves is seen in the distal central prostate.
b) A large nerve bundle containing l-Enk immunoreactive nerves is seen lying within smooth muscle in the distal central prostate.

a)



b)

Figure 8.4

NPY-immunoreactivity in sections of the human prostate (scale 1cm=30μm.)
a) A ganglion containing brightly fluorescent NPY-immunoreactive nerve cell bodies and a few nerve fibres is seen in the distal central prostate.
b) Beaded NPY-immunoreactive nerve fibres are seen running along the longitudinal axis of smooth muscle fibres of the anterior prostatic capsule.
c) A large nerve bundle containing NPY-immunoreactive nerve fibres is seen in the proximal central prostate.

a)

b)

c)

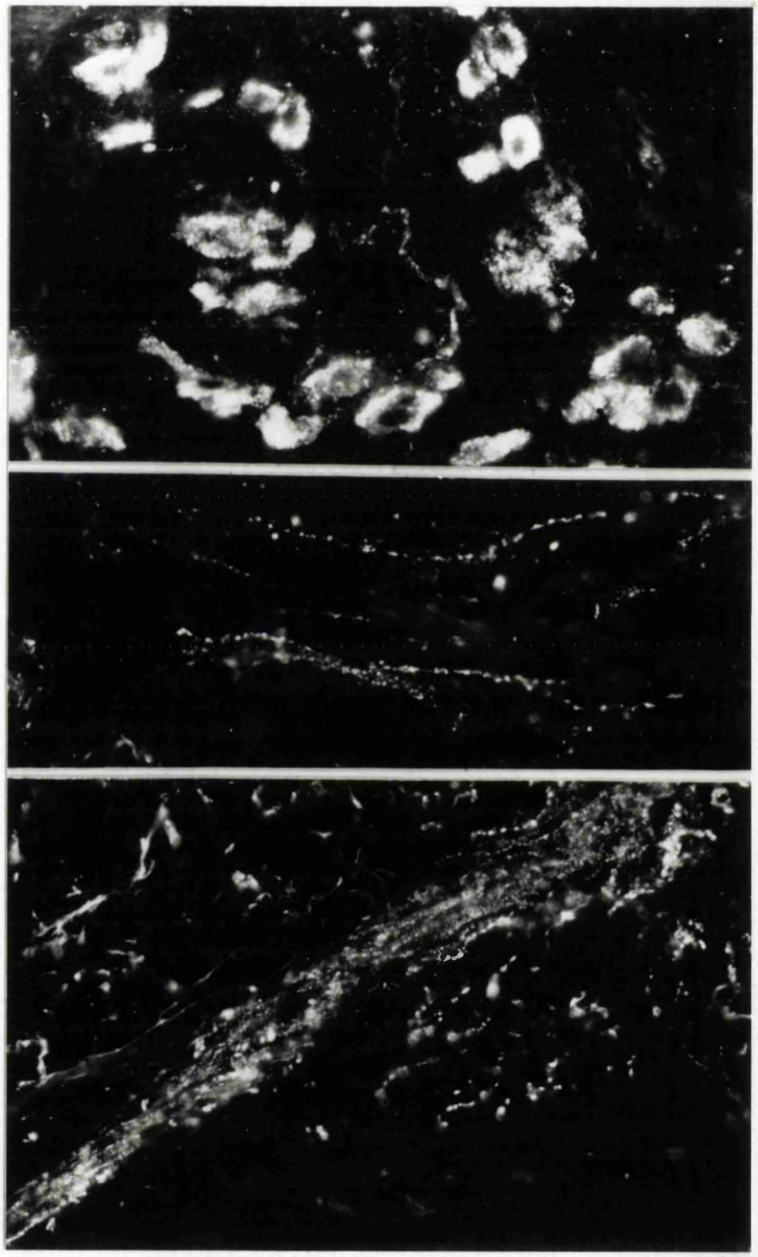


Figure 8.5

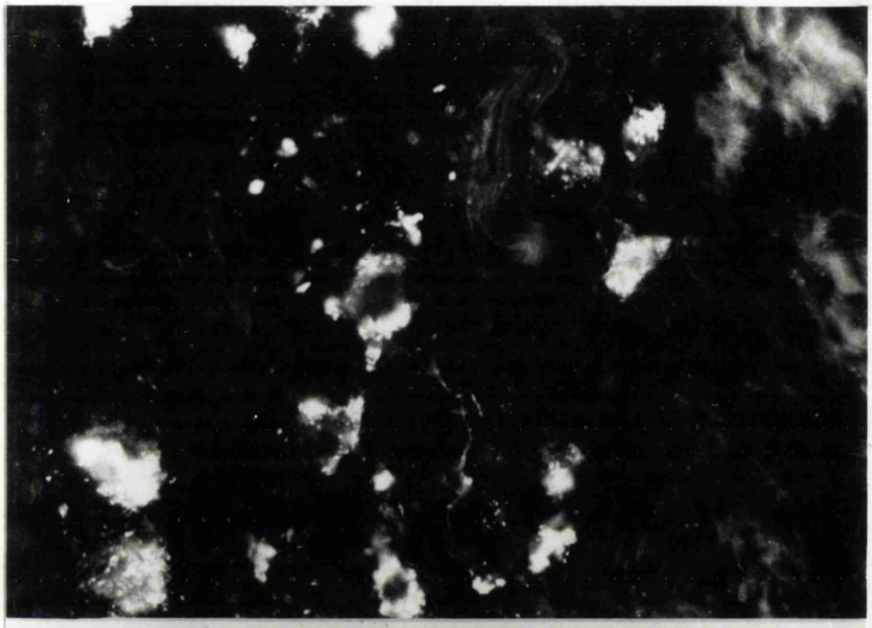
DBH-immunoreactivity in sections of human prostate (scale 1cm=30μm).

a) A ganglion containing DBH-immunoreactive nerve cell bodies and a few fluorescent nerve fibres are seen in the proximal central prostate. Most of the nerve fibres in this ganglion are non-fluorescent.

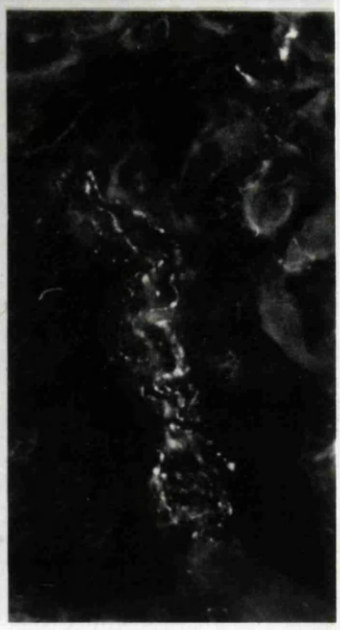
b) Fine, beaded DBH-immunoreactive nerve fibres are seen in the smooth muscle of the proximal central prostate.

c) A nerve bundle containing DBH-immunoreactive nerve fibres is seen in the proximal central prostate.

a)



b)



c)

Figure 8.6

VIP-immunoreactivity in sections of the human prostate. (scale 1cm=30μm).
a) A ganglion containing VIP-immunoreactive nerve cell bodies is seen in the anterior prostatic capsule. Collagen fibres are brightly autofluorescent.
b) Fine, beaded VIP-immunoreactive nerve fibres are seen in the smooth muscle of the proximal central prostate.

a)

b)

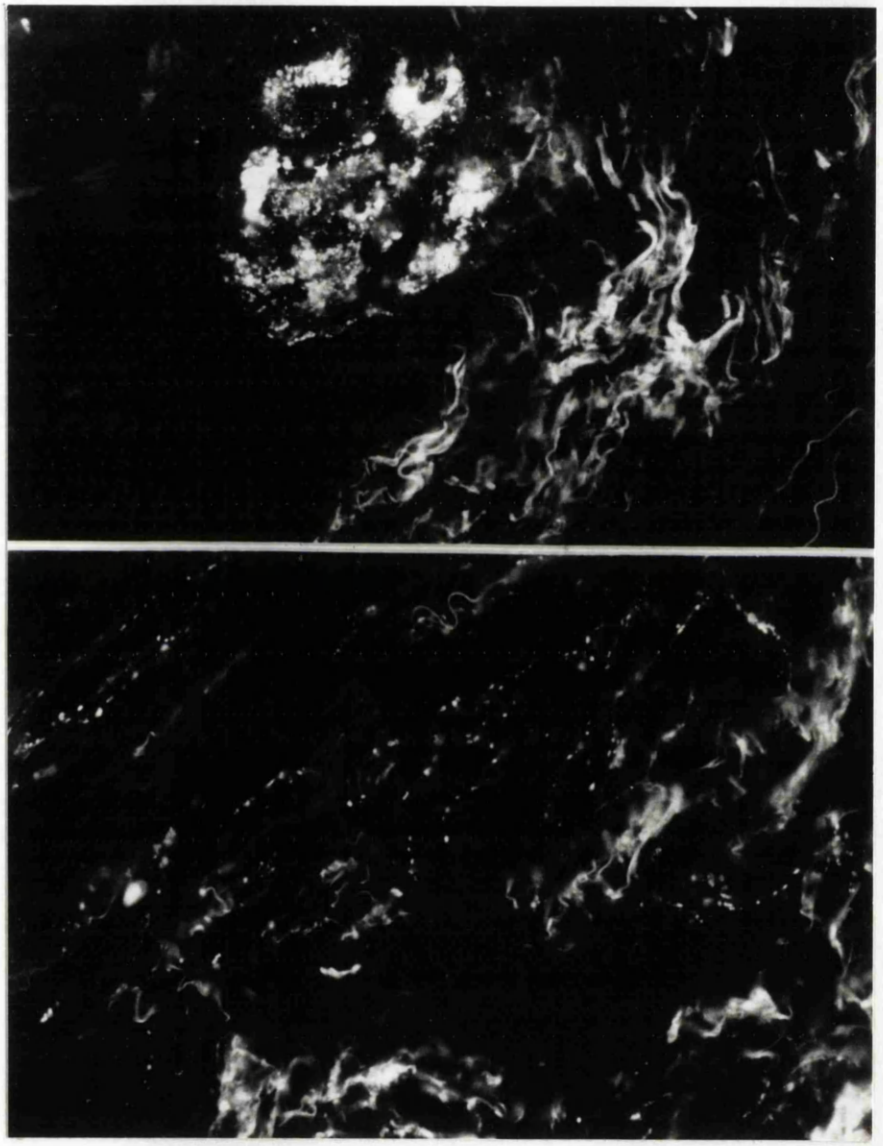


Figure 8.7

CGRP and substance P-immunoreactivity in sections of the human prostate (scale 1cm=30μm).

a) A ganglion containing CGRP-immunoreactive nerve cell bodies is seen in the distal central prostate.

b) A nerve bundle containing a few CGRP-immunoreactive nerve fibres is seen in the distal central prostate.

c) A ganglion containing substance-P immunoreactive nerve cell bodies and a prominent associated immunoreactive beaded nerve fibre is seen in the distal central prostate.

a)

b)

c)

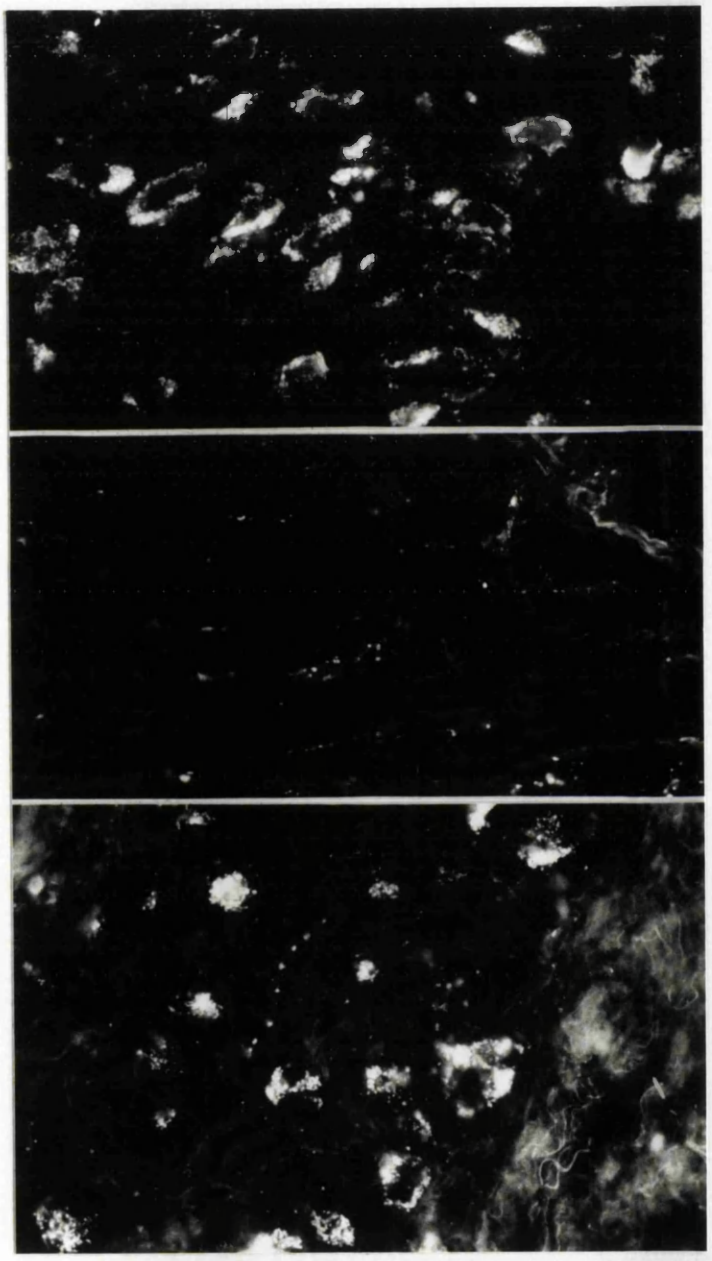


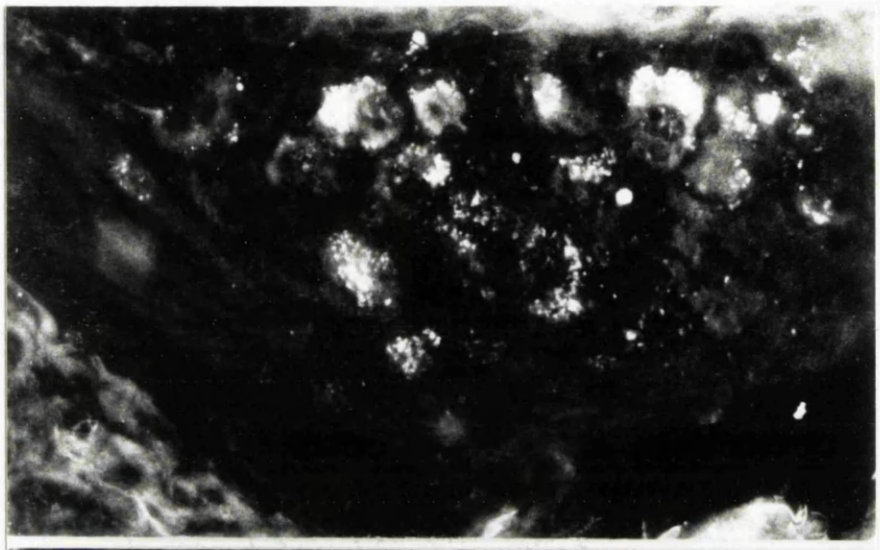
Figure 8.8

5-HT-immunoreactivity in sections of human prostate gland (scale 1cm=30μm).

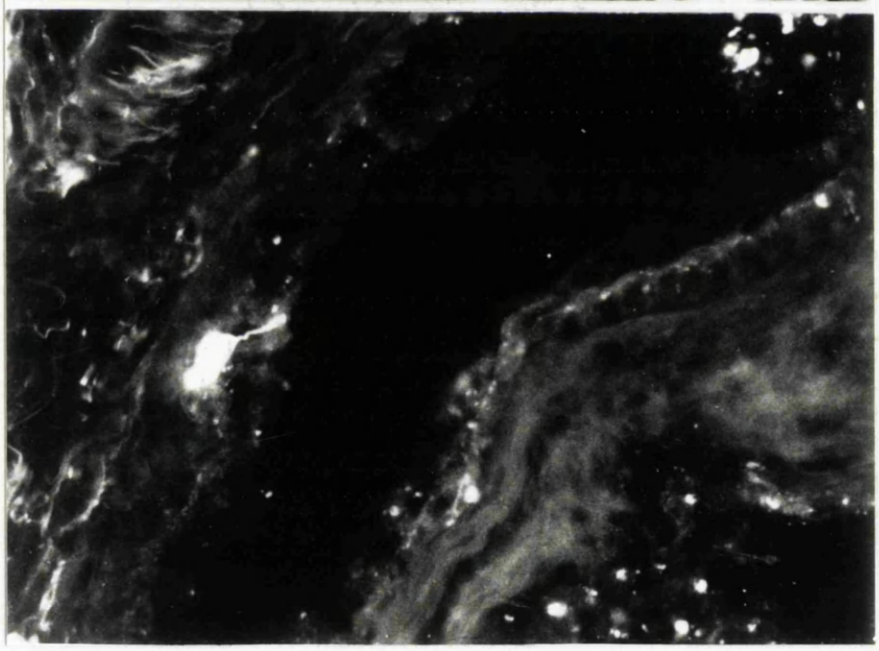
a) A ganglion containing 5-HT-immunoreactive nerve cell bodies is seen in the distal central prostate.

b) A 5-HT-immunoreactive *prostatic paracrine-endocrine cell*, located in tissue adjacent to a glandular acinus; note the dendritic process passing towards the acinar epithelium.

a)



b)



8.3.2 Histochemistry and Immunohistochemistry.

The prostate from patients without bladder outflow obstruction.

The intramural autonomic ganglia in all regions of the prostate contained DBH, AChE, 5-HT and all the peptides studied, except SOM. The greatest number of nerve cell bodies contained AChE and NPY followed (in decreasing number) by DBH, m-Enk, l-Enk, CGRP, VIP and 5-HT. Within prostatic ganglia nerve fibres were found to ramify between or encircle the nerve cell bodies (Figures 8.2 - 8.7) and contained DBH, AChE and all of the peptides studied except SOM, 5-HT and SP. 5-HT-immunoreactivity was absent from ganglia and positive nerves were the smallest neural population. High concentrations of 5HT-immunoreactivity were observed within certain cells of the prostatic glandular epithelial layer. Most of these cells had dendritic processes that extended between the epithelial cells (Figure 8.8).

In all regions of the prostate studied, most of the nerve fibres were found around alveoli and ramifying throughout the smooth muscle of the stromal compartment. They were found singly and/or in nerve bundles, running predominantly along the longitudinal axis of the smooth muscle fibres. The greatest concentration was of AChE-positive staining (see Table 8.1, Figure 8.9), followed in decreasing order of incidence by NPY, VIP, DBH, l-Enk, m-Enk, CGRP, SP and SOM (see Table 8.2, Figures 8.10, 8.11). All of these neural types occurred both as single nerves and running in fascicles. In contrast, CGRP-, SP- and 5-HT-immunoreactive nerves were seen predominantly within nerve bundles. NPY, VIP and DBH-immunoreactive

nerves were also observed on the adventitial/medial border of blood vessels.

Variation in neural staining might be expected to be least marked with AChE, because this neural type was present in the highest concentration and this would tend to reduce the influence of sampling errors. However, overall neural staining was very variable from one patient to another and could vary quite markedly within the tissue from an individual patient; therefore, a number of representative fields (minimum 10), were examined for each patient. Similar results were obtained using both the objective and subjective semi-quantitative techniques for AChE-staining nerves. AChE-stained nerves were thicker and exhibited a tendency to collect together in fascicles within the stromal compartment of the prostate with finer ramifications passing between acini. No apparent localisation of any particular peptide to a specific compartment was evident. Although the density of AChE-stained and VIP- and NPY-immunoreactive nerves remained similar in each region studied, that of the other nerve types varied in the different areas of the prostate studied.

In the proximal central prostate there were more SP, m-Enk, CGRP and DBH-immunoreactive nerves compared with the other prostatic regions, while in the distal central prostate there were fewer l-Enk, CGRP, DBH, 5-HT, and SP-containing nerves. In the peripheral prostate no m-Enk- and l-Enk-immunoreactive nerves were seen, and this region had the least number of DBH but the most dense innervation by 5-HT immunoreactive nerves. However, 5-HT-immunoreactive nerves were absent from the adjacent

prostatic capsule. Somatostatin-immunoreactive nerves were absent from the proximal central and peripheral regions but were occasionally observed in the capsule and distal central prostate.

The prostate from patients with bladder outlet obstruction

The overall distribution of nerve fibres and nerve cell bodies containing AChE, DBH, 5-HT and the peptides VIP, m-Enk, l-Enk, NPY, SP, CGRP, in the prostatic regions from patients with bladder outlet obstruction were similar to those in control patients. There was, however, a significant reduction in the density of these nerves in the obstructed bladder on subjective assessment (Figures 8.10, 8.11 and Tables 8.3, 8.4, 8.5).

An objective quantitative technique demonstrated this very clearly for AChE-positive staining (Figure 8.9 and Table 8.1). Statistical analysis suggested that these data were not normally distributed, therefore non-parametric analysis was carried out using the Mann Whitney U test. All of the obstructed groups exhibited a highly significant reduction in innervation as contrasted to control ($p<0.0001$). As mentioned previously there was significant intraprostatic variation, but this did not appear to follow any consistent pattern.

In all three obstructed groups, there was an increase of DBH- and l-Enk-immunoreactive nerves in the peripheral prostate, whilst in the group with acute urinary retention there was also an increase of VIP- and CGRP-immunoreactive nerves in this region. Most other prostatic regions from patients in all three obstructed groups showed a decrease in nerve density

when compared with control patients. There were however the following exceptions:

(i) In the stable obstructed patients, the densities of VIP-immunoreactive nerves in the anterior capsule and peripheral prostate; SP-immunoreactive nerves in the peripheral prostate and proximal central prostate and CGRP-immunoreactive nerves in the anterior capsule were the same as for the control group.

(ii) In the unstable obstructed group, the densities of VIP- and NPY-immunoreactive nerves in the anterior capsule, and CGRP-immunoreactive nerves in the proximal central prostate were the same as in controls.

(iii) In the acute retention group, the densities of SP- and NPY-immunoreactive nerves in the peripheral prostate and NPY-immunoreactive reactive nerves in the distal central prostate remained the same as controls.

Table 8.1

	A	B	C	D	E	F
CONT	110.7 ± 77.9 (n=3)	---- (n=0)	166.7 ± 67.5 (n=3)	114.7 ± 22.3 (n=3)	166.7 ± 35.7 (n=3)	144.7 ± 40 (n=3)
UNST OBS.	38.7 ± 28.1 (n=4)	36 ± 8.45 (n=4)	25 ± 10.3 (n=4)	24.7 ± 11.3 (n=4)	24.88 ± 9.81 (n=8)	27.7 ± 13.3 (n=4)
STAB. OBS.	22 ± 11 (n=2)	33.3 ± 11.3 (n=3)	44.5 ± 33.5 (n=2)	5.5 ± 5.5 (n=2)	22 ± 6.02 (n=5)	27.5 ± 16.5 (n=2)
AC. RET.	29.7 ± 13.5 (n=3)	19.25 ± 9.39 (n=4)	18.3 ± 13.2 (n=3)	11 ± 6.35 (n=3)	39.7 ± 14 (n=7)	29.33 ± 9.7 (n=3)

OBJECTIVE QUANTIFICATION OF ACETYLCHOLINESTERASE POSITIVE NERVES/mm² MUSCLE.

Results expressed as mean ± Standard error of mean (n = number of patients in each group).

CON = CONTROL
UNST.OBS. = UNSTABLE OBSTRUCTION
STABLE OBS. = STABLE OBSTRUCTION
ACUTE RET. = ACUTE RETENTION

Table 8.2

HUMAN PROSTATE

CONTROL

Prostatic Area	VIP	SP	SOM	mENK	NPY	CGRP	5HT	DBH	1ENK
Anterior Capsule	+++	+	+/-	+	+++++	++ NB	–	++++	+++
Peripheral Zone	+++	+	–	–	+++++	++ NB	++++	+	–
Distal	+++	+/-	+/-	+	+++++	+	+	+++	++
Proximal	++++	++ NB	–	+++	+++++	+++	+++	+++++	+++

Key

+	= sparse
++	= sparse/moderate
+++	= moderate
++++	= moderate/dense
+++++	= dense
NB	= Nerve bundle

Table 8.3

STABLE OBSTRUCTED

Prostatic Area	VIP	SP	SOM	mENK	NPY	CGRP	5HT	DBH	1ENK
Anterior Capsule	↔	↓	↓	↓	↓	↔	–	↓	↓
Peripheral Zone	↔	↔	–	–	↓	↓	↓	↑	↑
Centre	↓	↔	↓	↓	↓	↓	↓	↓	↓
Distal	↓	↓	–	↓	↓	↓	↓	↓	↓

Table 8.4

UNSTABLE OBSTRUCTED

Prostatic Area	VIP	SP	SOM	mENK	NPY	CGRP	5HT	DBH	1ENK
Anterior Capsule	↔	↓	↓	↓	↔	↓	–	↓	↓
Peripheral Zone	↓	↓	–	–	↓	↓	↓	↑	↑
Centre	↓	–	↓	↓	↓	↔	↓	↓	↓
Distal	↓	↓	–	↓	↓	↓	↓	↓	↓

Table 8.5

RETENTION

Prostatic Area	VIP	SP	SOM	mENK	NPY	CGRP	5HT	DBH	1ENK
Anterior Capsule	↓	↓	↓	↓	↓	↓	–	↓	↓
Peripheral Zone	↑	↔	–	–	↔	↑	↓	↑	↑
Centre	↓	↓	↓	↓	↓	↓	↓	↓	↓
Distal	↓	↓	–	–	↔	↓	↓	↓	↓

Figure 8.9

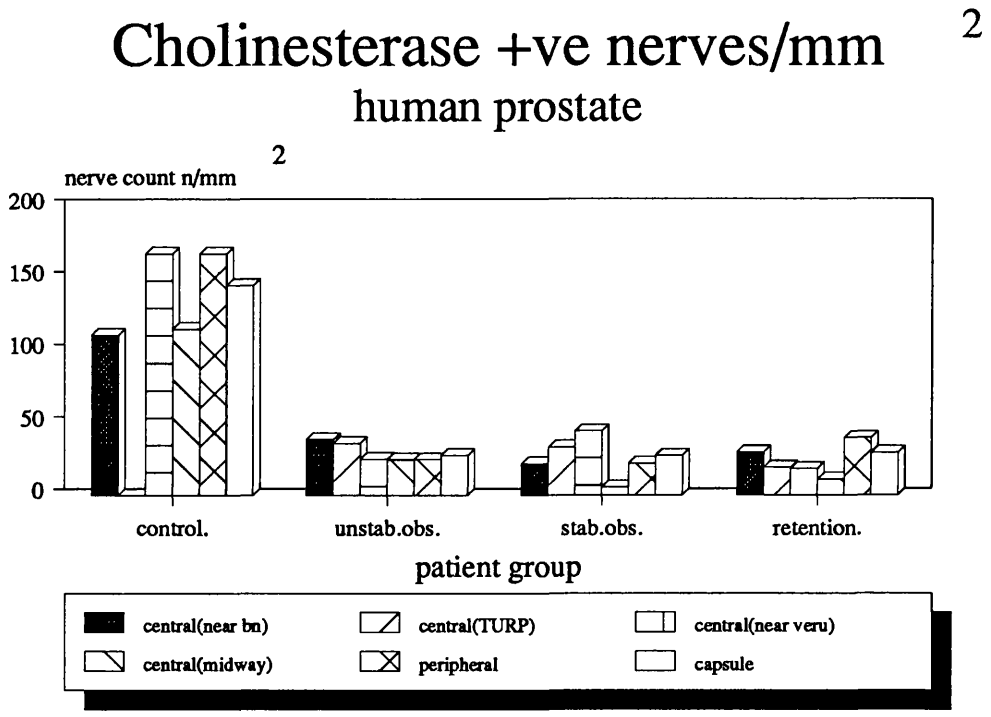
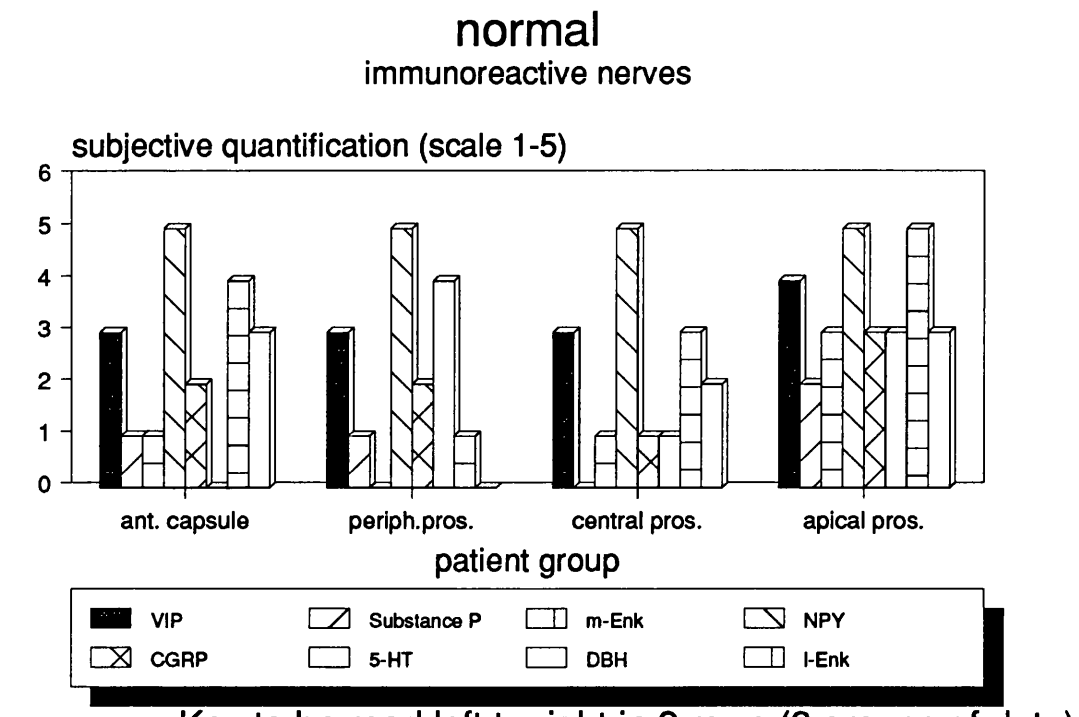
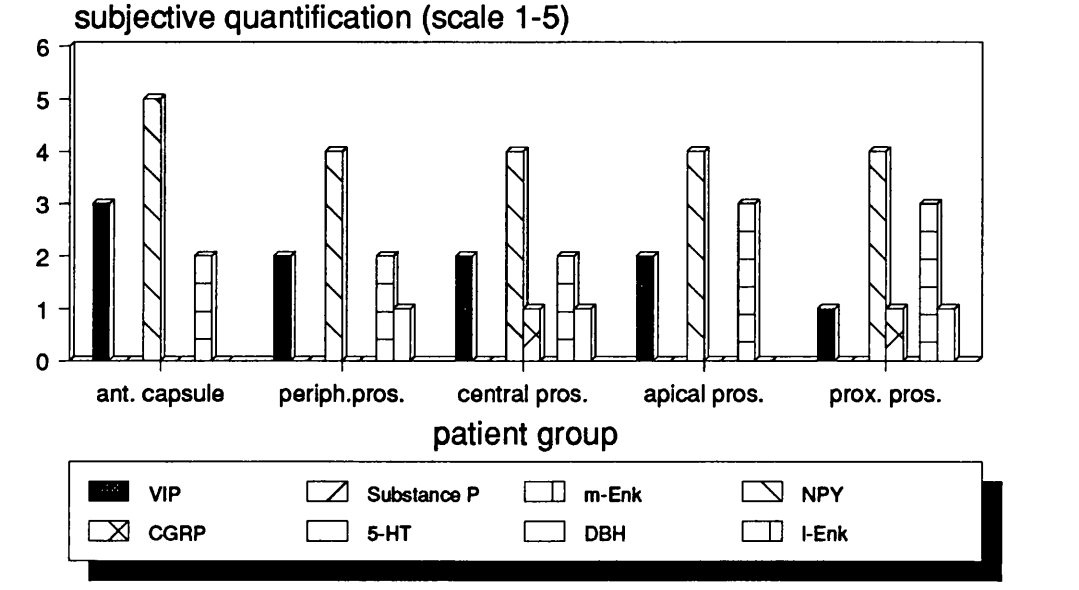

Cholinesterase +ve nerves/mm 2
human prostate
nerve count n/mm 2
200
150
100
50
0
control.
unstab.obs.
stab.obs.
retention.
patient group
central(near bn)
central(TURP)
central(near veru)
central(midway)
peripheral
capsule

Figure 8.10



Key to be read left to right in 2 rows (8 groups of data) for each region studied.

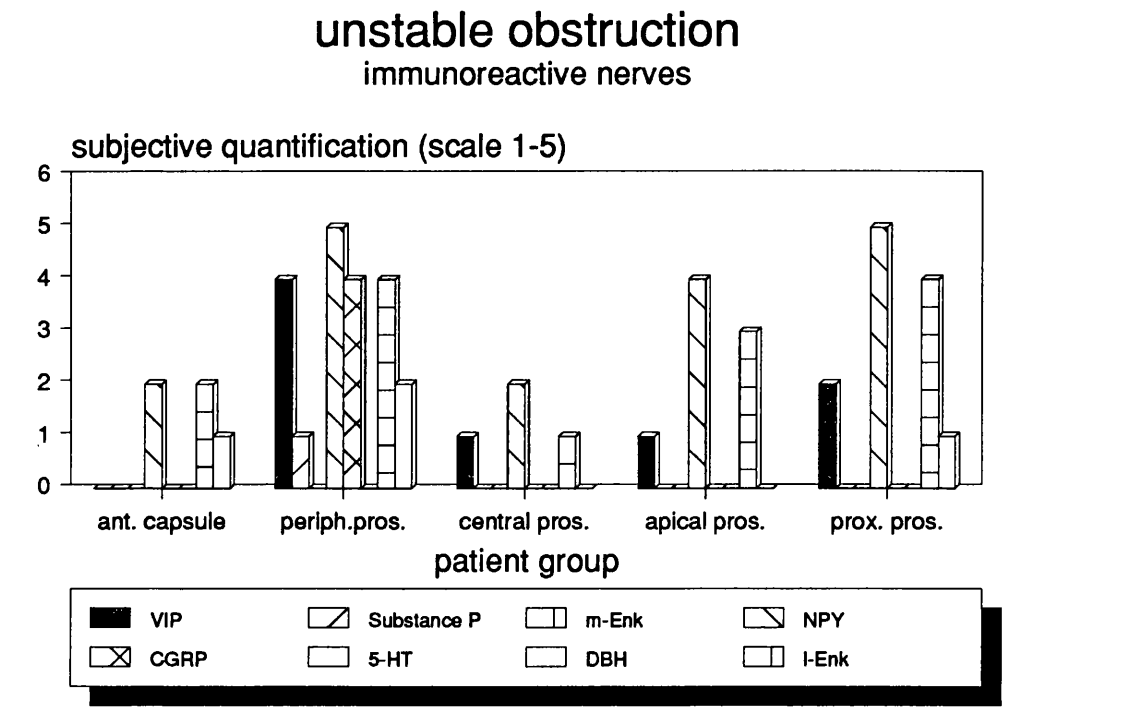
human prostate



Key to be read left to right in 2 rows (8 groups of data) for each region studied.

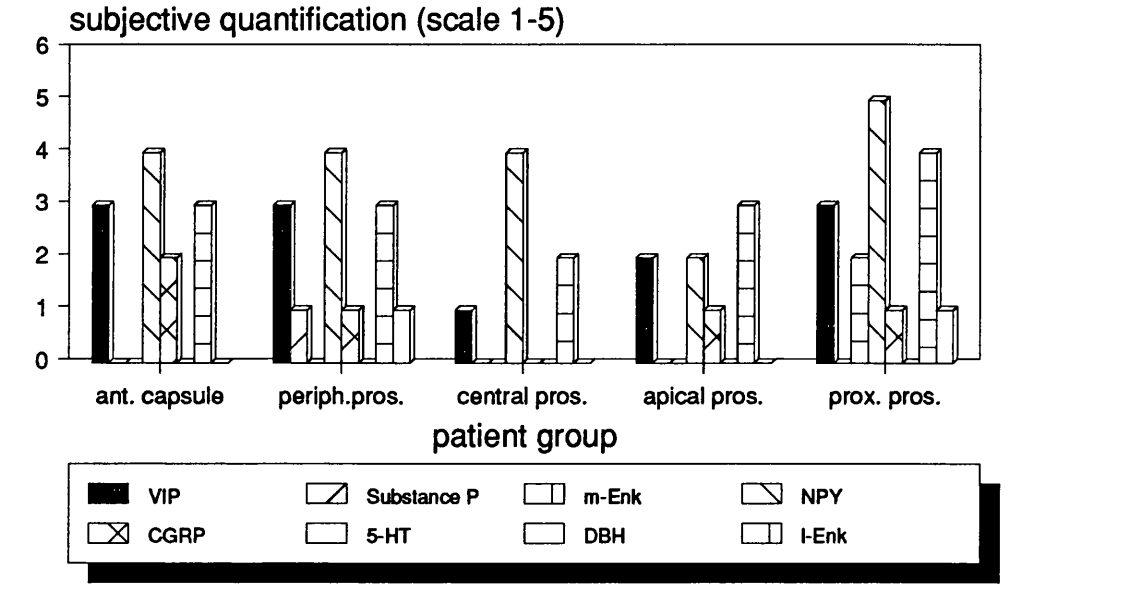
human prostate

Figure 8.11



Key to be read left to right in 2 rows (8 groups of data) for each region studied.

human prostate



Key to be read left to right in 2 rows (8 groups of data) for each region studied.

human prostate

8.4 DISCUSSION

In the present study, nerve fibres and nerve cell bodies containing neuropeptides, 5-HT, DBH and AChE have been described in the different regions of the human prostate. Care must however be exercised in interpreting these results. It must be remembered that AChE-positive staining may not be entirely specific for cholinergic nerves, since AChE has been reported to occur without acetylcholine in peripheral cell bodies on adrenergic ganglion cell bodies and on the membrane of some Schwann cells (Robinson 1971), is known to degrade peptides such as substance P (Chubb et al.1980) and may also stain sensory nerve fibres. Similarly the staining of noradrenergic sympathetic neurons can be extremely difficult and DBH is not a specific stain for adrenergic nerves.

In earlier studies, a decrease in the density of adrenergic and AChE-positive nerves has been reported in hyperplastic prostatic nodules in man (Dunzendorfer et al. 1976, Vaalasti and Hervonen 1980a). Indeed the altered innervation has even been proposed as a causal factor in the subsequent development of prostatic hyperplasia (Baumgarten et al. 1968). In the present study, although there was a great variation in the density of these various types of nerves in the different prostatic regions, a similar widespread reduction of AChE-stained, DBH- and peptide-immunoreactive nerves was observed in the prostate from patients with bladder outlet obstruction.

Blinded objective quantification of prostatic tissue using a point counting

technique revealed similar mean proportions of muscular and glandular tissue in both control prostate and that obtained from patients undergoing surgery for benign prostatic hyperplasia. Although tissue hyperplasia would tend to favour a reduction in nerve count, and would explain the patchy variation in innervation, the quantification technique used minimised errors due to this cause by a process of averaging. It therefore seems likely that the reduction in neural concentration was not solely a consequence of prostatic hyperplasia. We found no evidence of the abnormal, thin, coiled AChE-positive nerves described previously in the hyperplastic gland (Baumgarten et al. 1968).

This study confirmed previous observations (Vaalasti and Hervonen 1980a) in demonstrating a widespread and rather similar distribution of both sympathetic nerves (DBH-immunoreactivity) and parasympathetic nerves (AChE-positive staining) within prostatic smooth muscle. This would allow extensive neuronal interaction between the sympathetic and parasympathetic components of the autonomic nervous system, analogous to the situation described elsewhere in the lower urinary tract (Nergardh and Boreus 1972, Mutoh et al. 1987). Certainly, ganglia containing both AChE-positive staining and immunoreactivity to DBH and a number of putative peptide neurotransmitters were identified in all areas of the prostate. Previous workers have identified adrenergic terminals related to AChE-positive ganglia within the prostatic capsule (Kluck 1980) and electron microscopy studies have demonstrated the juxtapositioning of nerve fibres containing three

morphologically distinct types of axon profile which are thought to contain noradrenaline, acetylcholine and NANC neurotransmitters respectively (Vaalasti and Hervonen 1980b). Sympathetic stimulation induces expulsion of prostatic secretion produced and collected within prostatic acini, whilst parasympathetic stimulation is responsible for an increase in the rate of production of these secretions (Bruschini et al. 1978). It can be postulated that close synchronisation of the two components of the autonomic nervous system would allow increased activity in sympathetic neural pathways to increase prostatic secretion via a direct stimulation of parasympathetic nerves and conversely parasympathetic activity could increase the sensitivity of prostatic smooth muscle to sympathetic stimulation at the time of sexual arousal. The DBH-immunoreactive adrenergic innervation of blood vessels demonstrated here could presumably contribute by increasing blood flow to glandular tissue further to enhance secretory activity.

Previous workers have reported the presence of non-adrenergic non-cholinergic sensorimotor nerves in both the animal and the human prostate (Vaalasti and Hervonen 1979, Vaalasti et al. 1986), with reports of the presence of VIP and NPY (Alm et al. 1980, Gu et al. 1983b), with traces of somatostatin and substance P (Gu et al. 1983b) and met- and leu-enkephalin (m-ENK and l-ENK) (Vaalasti et al. 1980c). No previous studies have reported on a detailed investigation of the type and distribution of this NANC sensorimotor innervation within the human prostate gland. This study provides the first evidence that neuropeptides other than VIP are

present within intramural bladder ganglia. These findings constitute a histological basis which supports a potential interaction between sensorimotor nerves containing these putative neurotransmitters and classical autonomic nerves.

Although the neuropeptides and 5-HT could be demonstrated within nerves in all of the tissue compartments of the prostate (see figure 4), a predominant localisation of NPY to muscle and VIP with glandular acini, as previously reported (Higgins et al. 1989), was not apparent. The complete absence of any physiological or pharmacological data on the action of the neuropeptides and 5-HT within the human prostate renders their precise role the matter of speculation at present. Nevertheless, the number and wide-spread distribution of these putative neurotransmitters within prostatic nerves and the presence of numerous intramural ganglia suggests that these substances play an important functional role within the prostate gland.

NPY and VIP were the peptides present in the greatest concentration within the prostate. Both peptides have been reported to have motor functions elsewhere in the lower urinary and intestinal tract. In the human bladder VIP produces dose-related prolonged relaxation effects and a powerful vasodilator effect (Larsen et al. 1981, Said 1984). Similarly VIP-containing nerves in the penis have ben attributed a role in the control of penile erection and ejaculation, since exogenous VIP has an inhibitory effect on penile and vas deferens smooth muscle contraction (Larsen et al. 1981). NPY is known as a pre- and post-synaptic modulator of adrenergic

transmission and is a potent vasoconstrictor in some vessels (Lundberg and Tatemoto 1981). Studies carried out on other tissues have demonstrated that VIP is present within cholinergic neurons and NPY in adrenergic nerves (Lundberg and Tatemoto 1982, Lundberg 1981). Similarly, somatostatin, l-Enk and m-Enk, VIP and NPY have all been demonstrated in different populations of adrenergic nerves (Hokfelt et al. 1977, Shultzberg et al. 1980); the possible co-storage of VIP and acetylcholine in the human prostate is supported by the results of ultrastructural studies and immunoelectron microscopy (Gu et al. 1983b).

CGRP and SP-immunoreactivity has previously been associated with sensory nerves and these substances are thought to be important neurotransmitters in the mediation of pain and in axon reflexes causing vasodilatation in skin (Hokfelt et al. 1980). CGRP and SP are principally co-localised to sensory nerve fibres (Le Greves et al. 1985) and both substances have been demonstrated to exhibit other pharmacological effects. Substance P is known to produce contraction of detrusor and intestinal smooth muscle. CGRP often co-exists with SP within cholinergic neurons in the spinal cord and indeed has been reported to be a potent inhibitor of SP degradation (Le Greves et al. 1985). CGRP has been shown to both stimulate and relax smooth muscle in vitro and has a potent effect on blood vessels producing vasodilatation (Goodman and Iverson 1986). This study provides the first report of the presence of CGRP within the human prostate. It has to be concluded that whilst both SP and CGRP are likely to be fulfilling a

primarily sensory neurotransmitter role within the prostate, other potential roles cannot be excluded.

The endorphins and enkephalins are important in pain mechanisms and have been described previously in the human prostate (Vaalasti et al. 1980c), a finding confirmed by the study. Although both m-ENK- and l-ENK-immunoreactive nerves were absent in the peripheral prostate they were present in the other regions studied. The enkephalins inhibit synaptic transmissions in both the central and peripheral nervous system and are thought to influence both pre- and post-synaptic sites (North et al. 1979), subserving an important role in the central nervous system related to the control of micturition (de Groat and Kawatani 1985). At present it must be concluded that no evidence is available to support a substantive role for these agents in the local control of lower urinary tract function (Maggi and Meli 1986).

The greatest concentration of immunoreactivity to 5-HT was localised to cells within prostatic acinar tissue (see figure 8.8). These cells often exhibited dendritic processes which passed between adjacent cells. This cell type has been well described previously in the guinea pig (Hakanson et al. 1974) and human prostate and the descriptive term prostatic endocrine-paracrine (PEP) cell has been suggested (di Sant d'Agnese et al. 1985a). Current evidence based on the histological localisation of these cells within the glandular acini and adjacent to prostatic ducts suggests that they may play a neuroendocrine role, controlling the production and content of

prostatic secretion and acting as an additional modulator of the secretomotor action of the autonomic nervous system. 5-Hydroxytryptamine-containing PEP cells were reduced in hyperplastic as compared to normal prostate, a finding previously noted and suggested as being of potential importance in the pathogenesis of this condition (di Sant d'Agnese et al. 1984, 1985). In the presence of such a widespread reduction in innervation, it seems equally likely that the decrease in the PEP cells reflects a denervation phenomenon.

In man the prostate gland plays an important and complex role in reproductive physiology, and is the site of action of numerous hormonal influences, both systemic (sex hormones) and local (prostaglandins - originally described in this organ by von Euler 1931). In addition to the classical autonomic neurotransmitters acetylcholine and noradrenaline, a number of potential non-adrenergic, non-cholinergic neurotransmitters have been demonstrated in this histological study. Although the density of all of the neural populations was significantly reduced in the hyperplastic prostate, further progress however awaits pharmacological study to define their true functional role in both health and disease.

CHAPTER 9

PHARMACOLOGICAL CHARACTERISATION OF HUMAN PROSTATIC ADRENOCEPTORS.

9.1 INTRODUCTION

Benign hyperplasia of prostatic muscle and stromal tissues (BPH) is a well recognised age related phenomenon in the postpubertal male. Both clinical and postmortem studies have reported an incidence for BPH of at least 30% by the sixth decade (Hieble et al. 1986). The majority of patients with BPH do not seek medical advice. Even when troublesome symptoms ensue, the clinical course often remains static for many years and less than one-third of all patients who have prostatic symptoms actually require surgery (Ball et al. 1981). The management of prostatic bladder outflow obstruction in an ageing population is becoming an increasingly important part of routine urological practice. Although surgery will remain the mainstay of treatment in these patients, medical treatment has an important therapeutic potential. Such situations include:- patients on waiting lists for definitive surgery who require symptomatic relief during the waiting period; as a prophylactic measure in patients at risk of developing acute retention; and patients in whom surgery is either contraindicated or not indicated on objective grounds despite the presence of symptoms.

Recent work has suggested that nearly 50% of prostatic outflow obstruction, is mediated via the sympathetic component of the autonomic nervous system and is potentially reversible. The recognition of this *dynamic*

component acting via the stimulation of α_1 adrenoceptors (Furuya et al. 1982) has renewed interest in the therapeutic potential of α-adrenoceptor antagonists (Caine, 1986a,b). The importance of sympathetic influences is reinforced by the variations in symptom severity that accompany spontaneous modifications in sympathetic nerve traffic occasioned by cold and bladder distension and the observation that α-adrenoceptor agonists (in nasal decongestants and cold remedies) may induce acute retention in patients with prostatic symptoms. Initial experience with the α_1/α_2 antagonist phenoxybenzamine was disappointing due to the unacceptably high incidence of side effects. However, recent studies have sub-divided prostatic adrenoceptors into α_1 and α_2 subgroups (Lepor and Shapiro, 1984; Shapiro and Lepor, 1986). It has been suggested both on the basis of in vitro studies (Hieble et al. 1985) and clinical trials (Kirby et al. 1987) that stimulation of α_1 adrenoceptors is the main mechanism by which the sympathetic nervous system produces contraction of prostatic muscle (Kunisawa et al. 1985). Nevertheless, recent work using ligand binding techniques has reported an increase in the density of α_2 receptors as compared to α_1 receptors in the adenomatous prostate (Hedlund et al. 1985), a phenomenon for which no satisfactory explanation has so far been advanced. It was the intention of this combined receptor binding and isometric muscle strip study to resolve this paradox, in a multidisciplinary study, using pharmacological, biochemical and histochemical techniques.

9.2 MATERIALS and METHODS

Prostatic tissue specimens were obtained from 40 male patients with full informed consent, mean age 69 ± 2.2 years. Thirty-one of the patients underwent transurethral resection of the prostate and six underwent retropubic prostatectomy as surgical treatment of urodynamically proven bladder outflow obstruction. Three patients (ages 44, 66 and 80 years) underwent radical cysto-urethrectomy for carcinoma; they had not been treated with radiotherapy and were considered to be controls, there being no clinical, urodynamic or histological evidence of bladder outlet obstruction.

In the patients undergoing transurethral surgery the tissue resected from the lateral lobes was subdivided in an arbitrary fashion into central and peripheral components, using the following technique. A loop resection of the central adenomatous prostatic tissue was performed and the bladder washed out allowing collection of this tissue. This was followed by biopsy of the peripheral tissue in a similar manner. The prostate specimens were obtained from men with no recent exposure to drugs known to influence sympathetic neurons.

9.2.1 Functional Muscle Strip Studies

Strips of prostatic tissue approximately 1cm x 1mm were cut under the dissecting microscope allowing portions with a significant number of fibres running in one direction to be selected. They were then suspended in 15ml organ baths. The tissues were bathed in Krebs solution of the following composition (expressed in mM): NaCl 133, KCl 4.7, $NaHPO_4$ 1.3, $NaHCO_3$

16.3, $MgSO_4$ 0.61, glucose 7.8 and $CaCl_2$ 2.52 (Bulbring, 1953). The solutions were gassed continuously with 95% oxygen and 5% CO_2 and maintained at 37°C. The tissues were placed under an initial resting tension of 0.5g and allowed to equilibrate for 60 minutes. Changes in isometric tension were recorded by means of a force displacement transducer (Grass FT03C) and displayed on a Grass polygraph (model 7D).

Cumulative dose response curves were constructed for noradrenaline (α_1 and α_2 agonist), phenylephrine (α_1 agonist) and UK-14,304 (α_2 agonist). Prazosin and rauwolscine were employed as selective α_1 and α_2 adrenoceptor antagonists respectively and pA_2 values determined by the method of Arunlakshana and Schild (1959). The effectiveness of cholinergic stimulation using acetylcholine as an agonist was investigated. Electrical field stimulation was carried out using two platinum ring electrodes, suspended 1cm apart. Pulse trains (0.5msec duration, 60V) were delivered using a Digitimer D4030 and isolated stimulator DS2. The prostatic strips were stimulated over a range of frequencies (2-50Hz) with a 5 minute interval between each stimulation. The consequences of pre-incubation with prazosin (10^{-7} M) or atropine (10^{-7} M) for 30 minutes were investigated. Tetrodotoxin 10 µg/10mls was used to abolish all neurally mediated responses to electrical stimulation.

9.2.2 Radioligand binding studies- Homogenates

Prostatic tissue from each patient was minced and homogenized with a Polytran Homogeniser (maximum speed for 20 seconds) in ice-cold buffer

containing 50 mM Tris-HCL (pH 7.6 at 25oC). The homogenate was centrifuged at 39,000g for 20 minutes at 4°C, and the pellet resuspended in fresh buffer and recentrifuged twice more. The final pellet was resuspended in fresh 50 mM Tris-HCl buffer and stored at -70°C until used.

9.2.3 Saturation Analysis

Saturation analyses were performed at several different concentrations of both ^{3}H-prazosin (0.05-20 nM) and ^{3}H-UK-4,304 (0.1-50 nM), for estimating α_1 and α_2 adrenoceptors respectively. Several tissues were also examined using ^{3}H-rauwolscine to estimate α_2 adrenoceptors. Total binding was determined in 1.0 ml volume containing 800µl of membrane protein (300g/tube), 100µl of each ^{3}H-ligand at varying concen-trations, and 100µl of 50 mM Tris-HC1 buffer (pH 7.6). Non-specific binding was determined in 1.0 ml, containing 800µl of tissue homogenate, 100µl of 10^{-5} M phentolamine and 100µl of each ^{3}H-ligand at varying concentrations. Total and non-specific binding were determined in triplicate whenever possible. The assays were terminated by filtration through Whatman GF/B glass fibre filters, using a Brandel cell harvester. The filter discs were then rinsed with 3 x 3 mls of buffer (4°C). The glass filter discs were placed in 3 mls of scintillation fluid ("Ecoscint"). The scintillation vials were then counted in an LKB scintillation counter.

9.3 RESULTS

9.3.1 Functional muscle strip studies.

Noradrenaline and phenylephrine ($3x10^{-7}$ to $3x10^{-5}$ M) produced concentration dependant contraction of adenomatous, peripheral and normal prostate tissue preparations. The maximal response obtained with phenylephrine (α_1 agonist) was some 75% of that obtained with noradrenaline (α_1/α_2 agonist) (Figure 9.1). The α_2 adrenoceptor agonist UK-14,304 studied over a concentration range of 10^{-7} to 10^{-4} M was without effect on all the muscle strip preparations tested (Figure 9.1). Prazosin (specific α_1 antagonist) was a potent competitive antagonist of noradrenaline mediated contraction, producing parallel shifts to the right in the dose response curve but with no reduction in the maximum tension developed (Figure 9.2). A Schild plot of these results (Figure 9.3) confirms this with a pA_2 value of 9.1 and a slope of -0.9.

The α_2 adrenoceptor antagonist rauwolscine was less effective than prazosin in producing relaxation of prostatic strips that had been pre-contracted with noradrenaline. Rauwolscine applied at a concentration of 10^{-9} M resulted in only a small parallel shift to the right of the noradrenaline dose response curve, consistent with the known selective α_2 antagonist activity of this compound (Figure 9.4). However, at high concentrations (10^{-8} M and 10^{-7} M) the top of the noradrenaline dose response curve was shifted. This finding is consistent with a recognised action of rauwolscine as an α_1 antagonist when it is present in high concentration. A

Schild plot of these results revealed a pA_2 of 8.8 but with a poor slope (-0.7), inconsistent with a competitive action on α_2 adrenoceptors (Figure 9.5).

The effect of rauwolscine on the dose response curve to noradrenaline when applied to a preparation of adenomatous prostate is shown in Figure 9.6. Rauwolscine 10^{-7} M caused only a modest shift to the right of the dose response curve. However, when prazosin 10^{-7} M was added to the bath and the response to noradrenaline repeated, an additional substantial shift occurred suggesting that the response was mediated via α_1 adrenoceptors in this tissue. A similar study is shown in Figure 9.7 in which prazosin was added first and rauwolscine second. Rauwolscine caused only a modest additional shift to the right of the dose response curve confirming that α_2 adrenoceptor-mediated contraction of hyperplastic prostatic tissue was of minimal importance.

The application of acetylcholine did not produce consistent contractile responses. Contractile response to nerve mediated stimulation was largely abolished by pretreatment with tetrodotoxin, a similar result was produced by incubation with prazosin, whereas atropine had little effect (Figure 9.8).

9.3.2 Radioligand Binding Studies-Homogenates

Saturation analyses were carried out on tissue from adenomatous central prostate (7 patients), peripheral prostate (8 patients), and normal control prostate (3 patients). The results of these studies are summarised in Table 9.1 and Figure 9.9. The binding of ^{3}H-Prazosin and ^{3}H-UK14,304 was

saturable and in each case a single class of binding sites was identified, as confirmed by a linear Scatchard analysis plot. The equilibrium dissociation constants (K_D) ranged between 0.32 and 0.52 nM for α_1 receptors and 1.8 and 3.10 nM for α_2 receptors (^{3}H Rauwolscine gave similar results). In some tissues it was not possible to measure α_2 binding sites but α_1 receptors were always present in all of the tissues examined. All tissues contained a much higher density of α_1 adrenoceptors than α_2, therefore the detection of α_1 receptors was taken as a marker of tissue viability and the results for α_2 receptors were analysed. In contrast, if both α_1 and α_2 receptors were absent, then the results were not included in the analysis, as it was assumed that the tissue was damaged. The adrenergic receptor concentration (B_{MAX}) was calculated in fmoles/mg protein. The range of values measured was 61.1-79.4 fmoles/mg protein for α_1 binding sites and 12.1-36.1 fmoles/mg protein for α_2 binding sites. Figure 9.9 contains representative Scatchard analysis curves for peripheral prostate, but similar patterns were also obtained with both central and normal prostate. This is evidenced by the correlation coefficients for the representative curves; control prostate ^{3}H-prazosin r= -0.96, ^{3}H-UK14,304 r= -0.94; peripheral prostate ^{3}H-prazosin r= -0.97, ^{3}H-UK14,304 r= -0.89; central prostate ^{3}H-prazosin r= -0.98, ^{3}H-UK14,304 r= -0.92. The ratio of α_1 to α_2 receptors was approximately 2:1 in adenomatous central prostate, and 4:1 in peripheral non-adenomatous tissue. Statistical comparison of these data using the Mann Whitney U statistic confirmed a significant difference between α_1 and α_2 receptor binding sites in the peripheral prostate (p=

0.0014). Conversely the apparent difference between α_1 and α_2 receptors for central prostate failed to reach significance (p= 0.1252).

Figure 9.1.

Cumulative dose response curve to noradrenaline, phenylephrine and UK-14,304.
(x axis - drug concentration/moles. y axis - tension in muscle strip preparations/ grams).

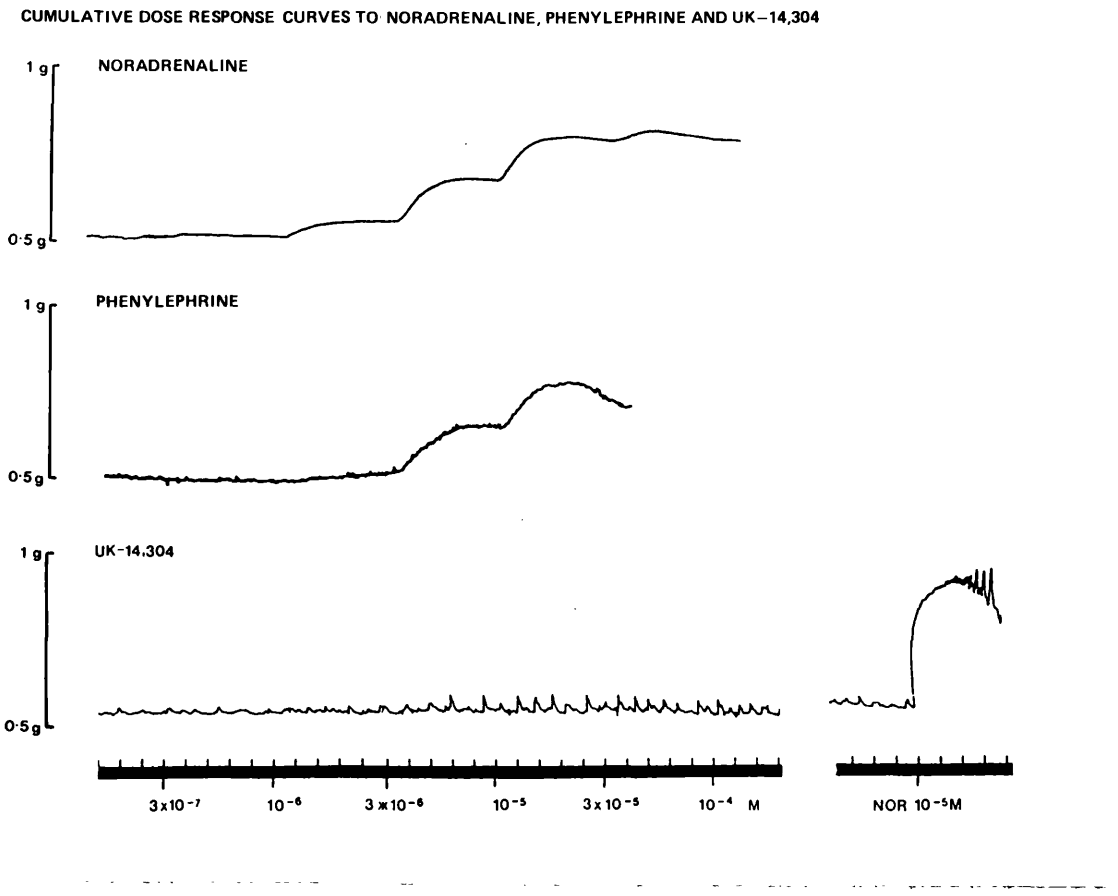


Figure 9.2.

Fitted dose response curves, contractile response to nor-adrenaline contrasting the response in the presence of differing concentrations of prazosin (10^{-9} M - 10^{-7} M).

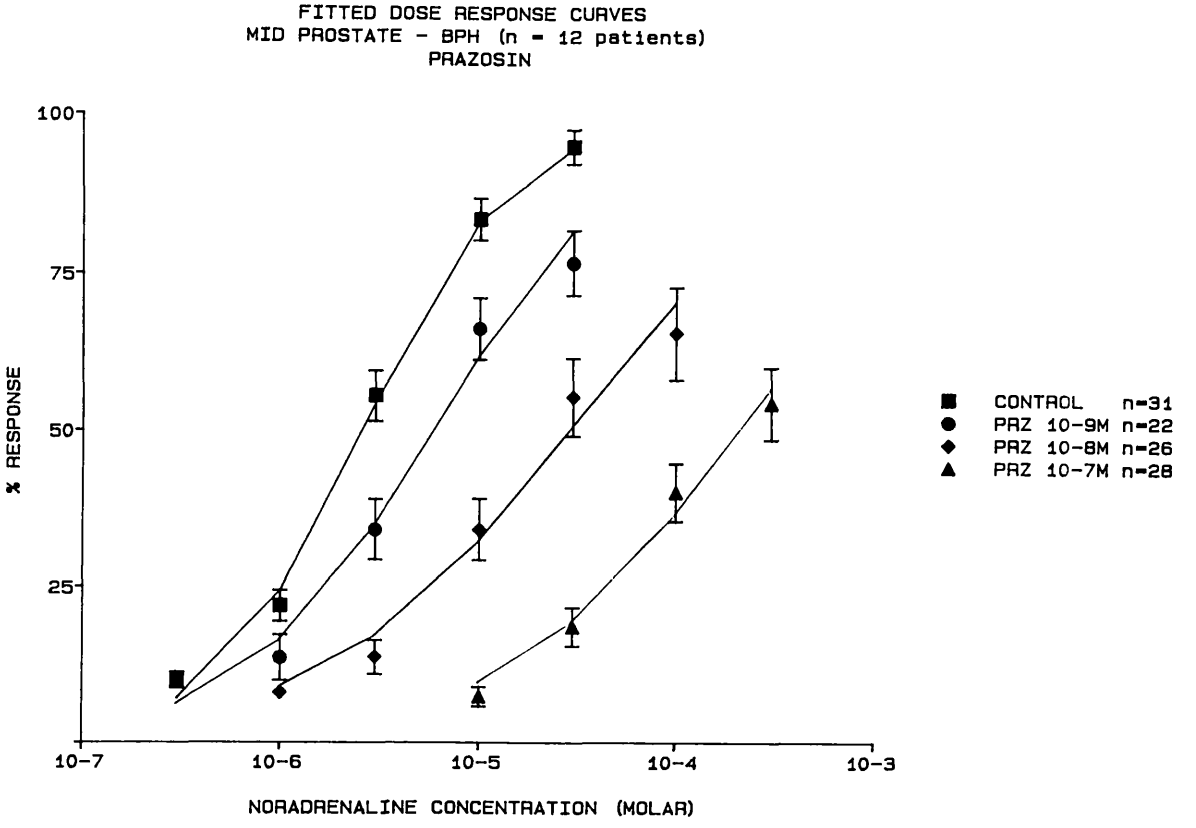


Figure 9.3.

Schild plot for prazosin in central prostate.

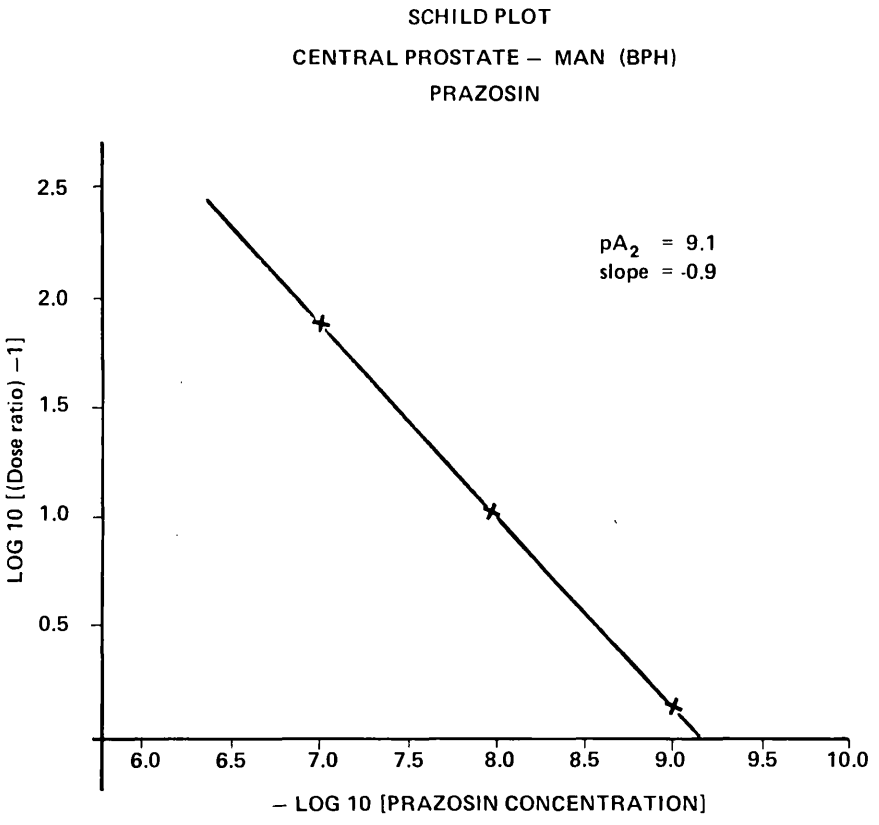


Figure 9.4.

Fitted dose response curves, contractile response to noradrenaline in the presence of differing concentrations of rauwolscine (10^{-9} M - 10^{-7} M).

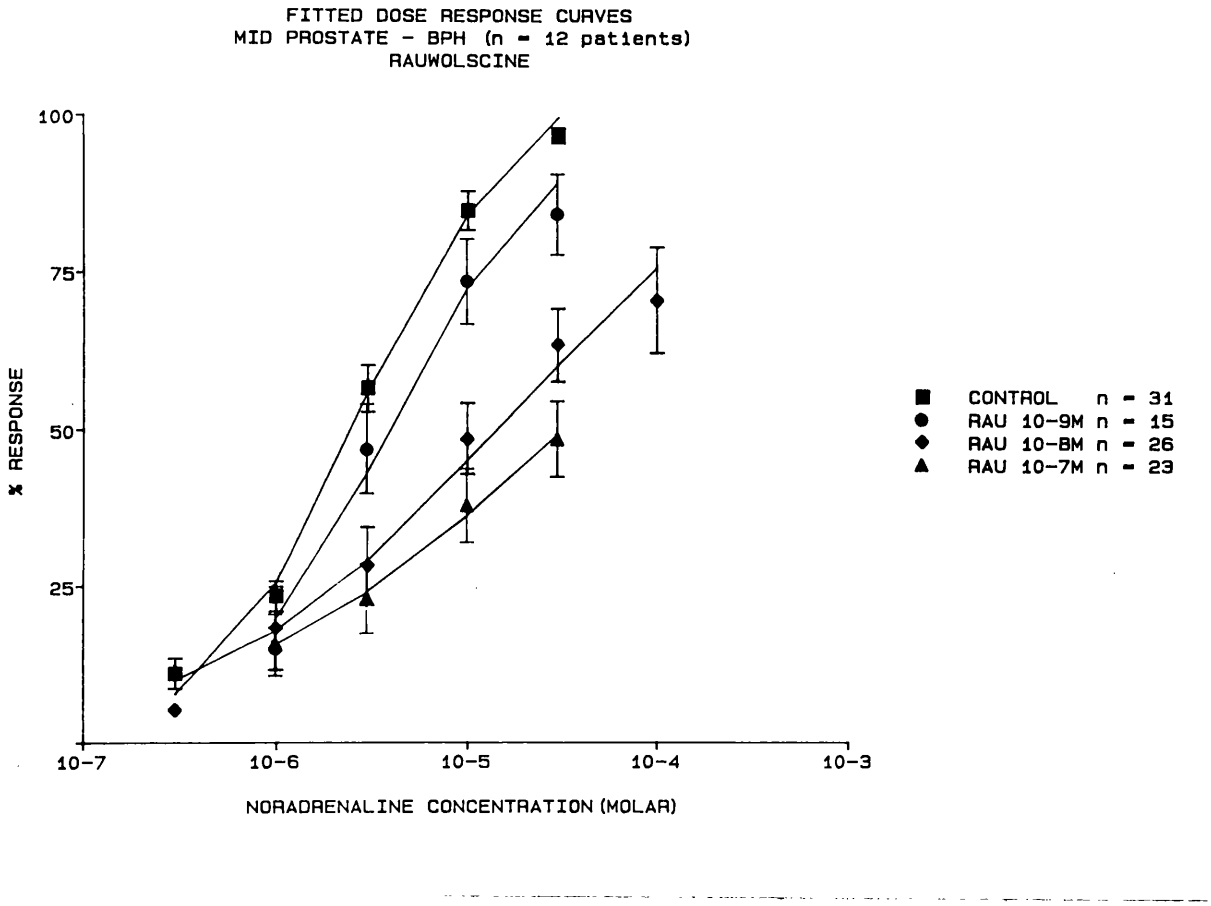


Figure 9.5

Schild plot for rauwolscine in central prostate.

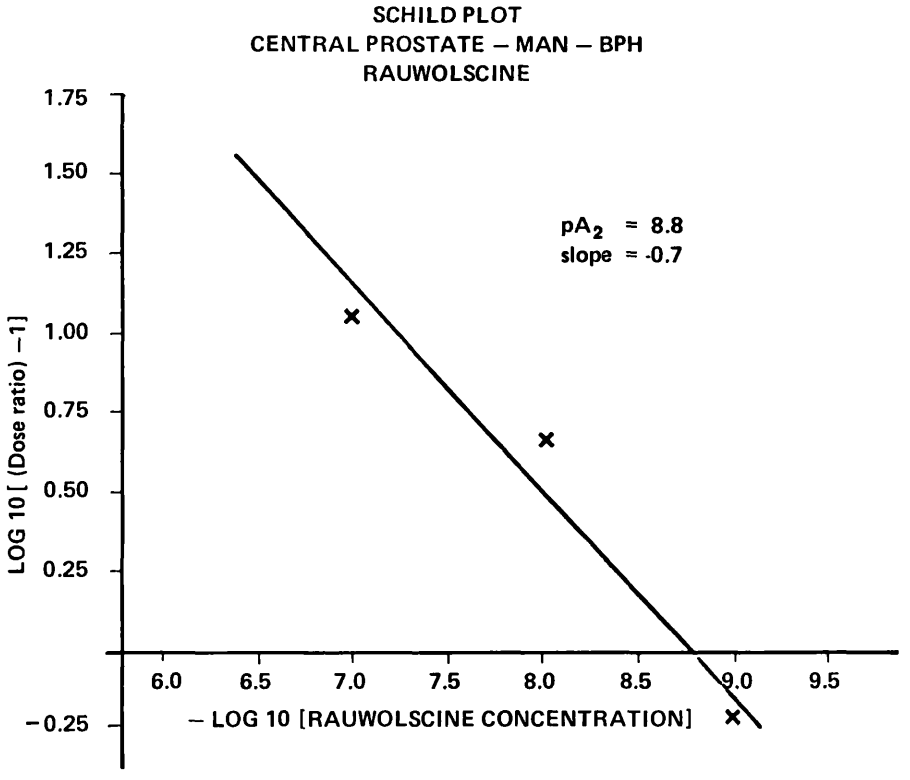


Figure 9.6

Fitted dose response curves contrasting the effect of rauwolscine 10^{-7} M and combined rauwolscine and prazosin 10^{-7} M on the contractile response to noradrenaline.

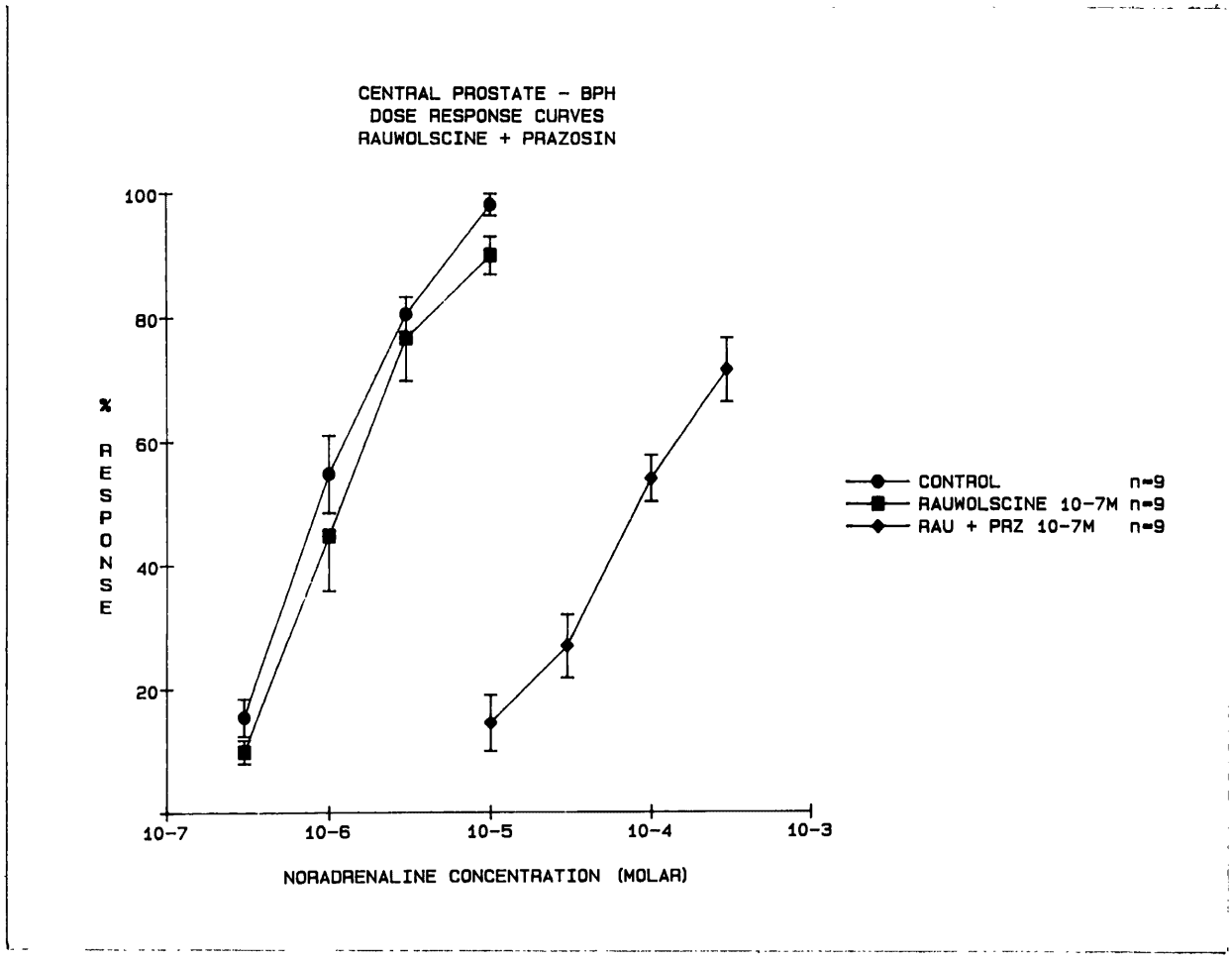


Figure 9.7.

Fitted dose response curves contrasting the effects of prazosin 10^{-7} M and combined prazosin and rauwolscine 10^{-7} M on the contractile response to noradrenaline.

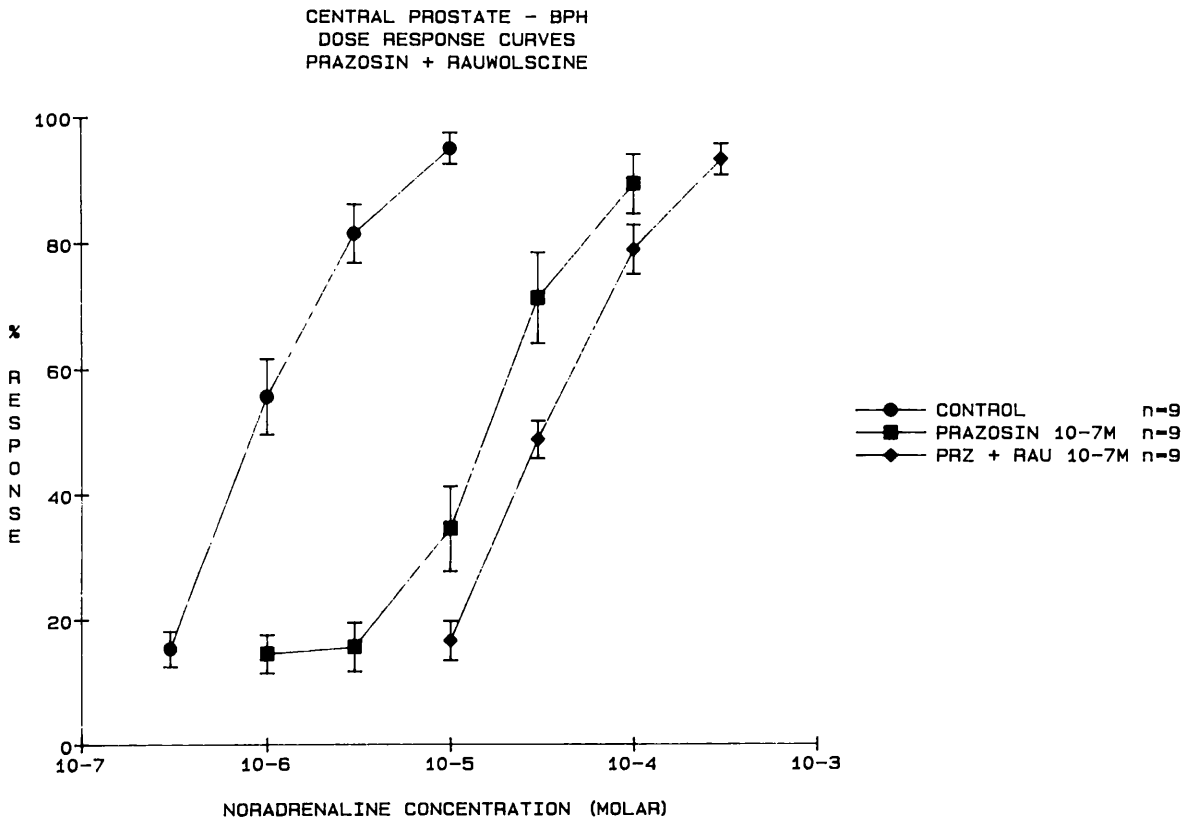


Figure 9.8.

a) Response to maximal stimulation with K^+.
b) Response to nerve stimulation.
c) Effect of pre-incubation with atropine $3x10^{-7}$ M on the response to nerve stimulation.
d) A comparison of pre-incubation with prazosin 10^{-7} M and TTX on the response to nerve mediated stimulation.

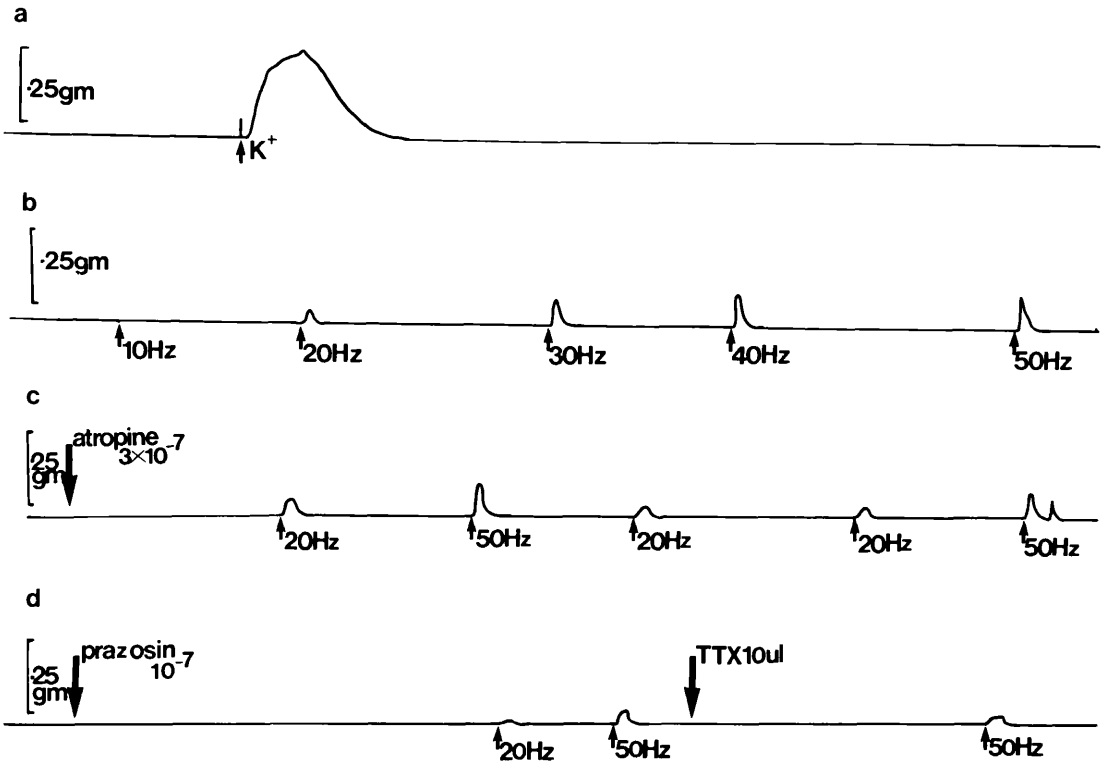


Figure 9.9.

Representative Scatchard plots for A) ^{3}H-Prazosin and B) ^{3}H-UK 14,304 binding to homogenates from 7 adenomatous prostatic tissues. Plots represent one experiment performed in triplicate and repeated 7 times on membranes obtained from different patients in each experiment. Equilibrium dissociation constant (KD), receptor concentration (Bmax) and linear correlation coefficient (LCC) values were determined from the Scatchard plots presented).

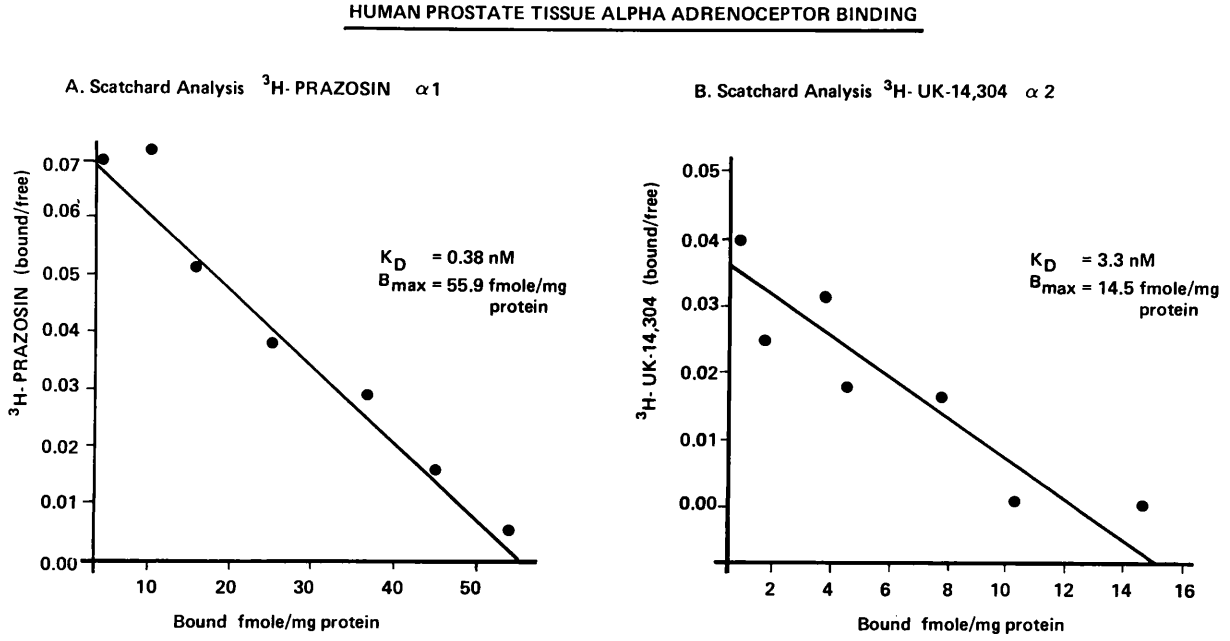


Table 9.1.

Summary of mean values ± standard error of mean for α adrenoceptor ligand binding studies (separate experiments performed in triplicate).

HUMAN PROSTATE TISSUE: Alpha-adrenoceptor Ligand Binding Studies

Sample	^{3}H PRAZOSIN $\alpha 1$		^{3}H- UK-14,304 $\alpha 2$	
	K_D (nM)	B_{max} (fmoles/mg)	K_D (nM)	B_{max} (fmoles/mg)
Prostate (BPH) (central) n = 7	0.51 ± 0.07	65.9 ± 5.2	2.34 ± 0.26	36.1 ± 6.2
Prostate (BPH) (peripheral) n = 8	0.32 ± 0.03	79.4 ± 11.1	3.10 ± 1.0	18.4 ± 3.1
Prostate (Control) n = 2	0.52	61.1	1.8	12.1

The above results represent the mean ± S.E.M. from separate experiments performed in triplicate.

9.4 DISCUSSION

Learmonth (1931) reported that stimulation of the pre-sacral nerve in man contracted the prostatic musculature. Since then, urethral pressure profile studies (Donker et al. 1972) and in vitro isometric prostatic strip studies (Caine, 1986a) have convincingly confirmed the important influence of the sympathetic nervous system on the bladder outflow tract, and raised the possibility of pharmacological treatment by α blockade. Initial experience with the non-selective α antagonist phenoxybenzamine was disappointing because of the unacceptably high incidence of side effects, attributable to the blockade of α_2 receptors and subsequent interference with the negative feedback control of the release of noradrenaline. With recognition of the importance of the α_1 receptor in mediating sympathetic action in the normal and adenomatous prostate, attention has turned to the therapeutic use of selective α_1 antagonists such as prazosin (Shapiro et al. 1981), with the intention of reducing unwanted side effects. Although numerous pharmacological isometric studies have been reported in the literature, only one study has also investigated normal prostatic tissue (Hedlund et al. 1985). Ligand binding techniques have only recently been applied to the investigation of human prostatic α_1 and α_2 adrenoceptors. These studies have raised an as yet unexplained paradox; one study demonstrating an excess of α_2 over α_1 receptors in adenomatous prostate despite a demonstrable functional predominance of α_1 receptors (Hedlund et al. 1985) and the other suggesting equivalent densities for both groups of α receptors (Lepor and Shapiro, 1984;

Shapiro and Lepor, 1986). It has been suggested that α_2 receptors may have an important role in mediating contraction of canine prostatic muscle (Shapiro et al. 1987), but care needs to be exercised in the extrapolation from animal models to man.

The results of the isometric studies reported here confirm that there is a functional predominance of α_1 adrenoceptors in human prostatic muscle. The limited inhibition by α_2 antagonists of in vitro prostatic muscle contraction confirms these findings and we report the first use of the specific α_2 agonist (UK14,304) on human prostatic tissue. The results of radioligand studies confirm the trend of an increase in α_2 binding sites towards parity in adenomatous tissue reported by previous studies, but demonstrate an overall predominance of α_1:α_2 receptors of approximately 3:1 which is in accordance with the results of the functional studies. A potential source of error would be if there were significant differences in innervation and histological structure between different areas of the same gland. We have carefully investigated this possibility by examining tissue from a number of different areas within prostatic adenomata removed at the time of open operation and, although there was significant regional variation, this appeared to be occurring randomly with no clearly identifiable regional trends.

This study by using a combination of investigative techniques has convincingly demonstrated the pathway whereby sympathetic stimulation produces prostatic muscular contraction. These results provide a scientific

basis for the use of α_1 adrenoceptor antagonists in the provision of symptomatic relief to selected patients with benign prostatic hyperplasia. Further work should investigate the existence of prostate specific α_1 adrenoceptors, which will allow the further refinement and tailoring of more effective symptomatic treatment for prostate obstruction.

CHAPTER 10

AUTORADIOGRAPHIC ANALYSIS OF ALPHA-ADRENOCEPTORS AND MUSCARINIC CHOLINERGIC RECEPTORS IN HYPERPLASTIC HUMAN PROSTATE.

10.1 INTRODUCTION

Mechanical and dynamic factors both seem to be important in the pathogenesis of bladder outflow obstruction due to benign prostatic hyperplasia (BPH). The dynamic component may vary rapidly according to the level of sympathetic stimulation acting on the prostate gland (Furuya et al. 1982). As demonstrated in the previous chapter, functional isometric prostatic muscle strip experiments have suggested that in both the normal and hyperplastic prostate, there is a functional predominance of α_1 adrenoceptors, and that noradrenaline-induced contraction in isolated preparations from the human prostate capsule and adenoma are effectively inhibited by prazosin. Clinical studies have confirmed that prazosin therapy significantly increases the urinary flow in men with BPH (Kirby et al. 1987, Chapple et al. 1989).

Previous radioligand binding experiments have identified and characterised α_1 and α_2 adrenoceptors in human prostatic adenomas (Hedlund et al. 1985, Lepor and Shapiro 1984, Lepor et al. 1987, Shapiro and Lepor 1986), but with conflicting results. Whereas Shapiro and Lepor (1986) suggest that the mean total density of α_1 and α_2 adrenoceptors in human prostatic adenomas are not statistically different, Hedlund et al. (1985)

propose that there are in fact more α_2 adrenoceptors in adenomatous tissue than α_1. These reports conflict with the studies reported in Chapter 9, which suggest that the α-adrenoceptors on prostatic smooth muscle are predominantly of the α_1 subtype.

The information obtained from binding experiments is limited. This is unavoidable as the actual cellular content of the tissue homogenate used is variable and unidentifiable. Use of an in vitro autoradiographical technique enables visualization of specific receptors within the different cellular compartments of the tissues studied, because the tissue morphology is maintained.

Recently, muscarinic cholinoceptors have been visualized and located predominantly on the prostatic epithelium (Hedlund et al. 1985, Lepor and Kuhar 1984). This is consistent with the suggested mechanisms underlying prostatic secretion (Bruschini et al. 1978, Smith et al. 1966). The present study represents the first localization of α_1 and α_2 adrenoceptors to specific human prostatic compartments, thus enabling a comparison to be made between function and location of these specific receptors.

10.2 MATERIALS AND METHODS

Radioligand Receptor Binding Methods: Slide-mounted tissue sections

10.2.1 Tissue preparation.

Portions of fresh prostate gland obtained from 10 men (mean age 69.5 ± 2.35 years) with BPH were freed of fat and connective tissue and placed directly onto cold microtome chucks, mounted in "Tissuetek" and frozen in liquid nitrogen-cooled isopentane. Serial sections were cut (10 µm) on a cryostat and mounted on acid-washed, subbed microscope slides. Sections were air-dried for 2 h and then stored at 4°C.

10.2.2 Autoradiography

The slide-mounted tissue sections were brought to room temperature. Labelling conditions for the receptor ligands were: ^{3}H-QNB (1 nM), incubation for 2 h in phosphate-buffered saline (PBS) at pH 7.4, two washes for 5 min at 4°C and a rinse in distilled water. Non-specific binding was determined using 1 µM atropine: ^{3}H-rauwolscine (2 nM), preincubation for 30 min in phosphate-buffered Krebs solution at pH 7.4, containing 10^{-5} M phenylmethylsulphonylfluride (PMSF), incubation in the same buffer for 1 h, two washes for 5 min at 4°C and a rinse in distilled water. Non-specific binding was determined using 10^{-5} M phentolamine: ^{3}H-prazosin (2 nM), incubation for 30 min in 170 mM Tris/HC1, pH 7.6 containing 10^{-5} M PMSF, one wash for 1 min at 25°C in buffer, followed by two 5 min washes at 4°C in the same buffer, and a final brief rinse in distilled water. Non-specific

binding was determined using 10^{-5} M phentolamine. They were then stored at 4°C overnight in a desiccator.

All labelling was carried out at 25°C, and labelled sections were placed in apposition to nuclear emulsion-coated coverslips (Young and Kuhar 1979, Ilford K2), and exposed at 4°C for 12-20 weeks (^{3}H-prazosin, ^{3}H- rauwolscine) or 5-10 weeks (^{3}H-QNB). After exposure, autoradiographic images were developed in D19 and fixed with Hypam. The sections were stained with toluidine blue, whilst ensuring that the autoradiographic coverslips were not displaced.

10.2.3 Analysis of binding.

The autoradiographs were examined by two independent observers using an image analysis system (Seescan) and, at the end of the study, the results were combined. The object of the analysis was to compare grain densities within a unit area consisting of only stromal cells, for the adrenoceptor radioligands. Serial sections from 10 patients were labelled with ^{3}H-prazosin and ^{3}H-rauwolscine (and with ^{3}H-QNB), thus enabling the same tissue area to be analyzed and compared for the two adrenoceptor ligands. This minimized errors introduced if stromal areas of different muscle cell content had been measured. Also, comparison was limited to individual patients, thereby reducing the error due to variation in cell size and innervation. As the image analysis system used was linked directly to the microscope, the definition lost on photographic analysis of the autoradiographic images was minimized. The autoradiographic grains and the background within a unit

area on the image analysis screen were distinguished on the basis of colour (dark green autoradiographic grains on a light green background). The percentage area occupied by dark green grains was measured. For both radioligands, mean total and non-specific binding values (% grain occupancy /unit area) were determined for each stromal area from each prostate section, for both radioligands. Specific binding values for each radioligand and each patient were obtained by subtracting the non-specific binding values from the total binding values. Specific binding values for each patient and radioligand were compared, allowing ratios of α_1 and α_2 adrenoceptors respectively to be calculated for the prostatic stroma of each patient.

10.2.4 MATERIALS

^{3}H-prazosin, specific activity 82 Ci/mmol (New England Nuclear); ^{3}H-rauwolscine, specific activity 88.7 Ci/mmol (New England Nuclear); 3H/UK 14,304, specific activity 85.5 Ci/mmol (New England Nuclear); ^{3}H-QNB, specific activity 39 Ci/mmol (Amersham International); phentolamine hydrochloride (rogitine, Ciba-Geigy); phenylmethylsulphonylflouride (Sigma); atropine sulphate (Antigen Ltd.).

10.3 RESULTS

The autoradiographic images obtained from the three different radioligands used illustrated the contrasting localizations of the three binding sites. An image analysis system (Seescan) was used to examine each autoradiographic slide (see Figure 10.1) in order to quantify and compare the grain distributions seen with ^{3}H-rauwolscine and ^{3}H-prazosin in the stromal region of each specimen (n=10). The mean specific binding value (% grain occupancy /unit area) in the stroma of each prostate specimen was calculated for both adrenoceptor radioligands used (Table 10.1). The mean specific binding values for the α_1 and α_2 adrenoceptors were 4.5 ± 0.39 and 1.6 ± 0.37 respectively, and the average ratio of α_1: α_2 binding sites in the prostatic stroma was 3.9 ± 0.75. The differences between these two groups were very highly significant (Mann Whitney $p<0.0001$). This predominance of α_1 over α_2 adrenoceptors in the human prostatic stroma, as quantified by image analysis, is illustrated in Figure 10.2.

The majority of ^{3}H-prazosin binding identified was confined to the stroma. In comparison, a sparse grain distribution was seen with ^{3}H-rauwolscine in the same region, but extensive labelling was seen on the numerous blood vessels present in these tissue sections. Figure 10.3.(d-f) illustrates that whilst a dense distribution of α_1 adrenoceptors was seen in the stroma, only sparse labelling was apparent on the blood vessel. In contrast, a dense α_2 adrenoceptor population was seen on the same blood vessel (consecutive sections), but little binding was seen in the stroma.

This study also confirms the location of muscarinic cholinoceptors on glandular epithelium, as reported in previous studies; Figure 10.3.(a-c) clearly illustrates the different locations of α_1 and muscarinic cholinergic binding sites to the stroma and glandular epithelium respectively. In addition to extensive blood vessel labelling, a population of α_2 adrenoceptors was seen in close proximity to the base of some of the ^{3}H-QNB-labelled glandular epithelial cells (Figure 10.4). The distribution of α_2 adrenoceptors in human adenomatous prostatic tissue is summarized in Figure 10.5.

The level of non-specific binding seen with ^{3}H-prazosin (as defined after displacement with 10 µM phentolamine) was occasionally high and varied from specimen to specimen (range: 10-40%). For both ^{3}H-rauwolscine and ^{3}H-QNB however, the non-specific binding (as defined by displacement with 10 µM phentolamine and 1 µM atropine respectively) was uniformly negligible.

Table 10.1

Specimen number	Specific receptor binding *		Approximate ratio Alpha-1 / Alpha-2
	Alpha-1	Alpha-2	
1	5.9	1.1	5:1
2	2.2	1.5	3:2
3	3.9	1.6	5:2
4	3.4	0.8	9:2
5	5.0	1.0	5:1
6	5.6	4.5	5:4
7	4.2	0.6	7:1
8	4.7	2.6	2:1
9	6.2	0.8	8:1
10	3.6	1.8	5:2
Mean ± S.E.M.	4.5 ±0.39	1.6 ±0.37	3.9 ±0.75

* % grain occupancy/unit area

Table 10.2

Binding site	Basal lamina	Epithelium	Stroma (smooth muscle)	Blood vessels
α_1	—	—	*** [*]	*
α_2	***	—	**	****
M	—	****	*	—

Figure 10.1

Consecutive serial sections of human prostate (magnification x 60) are shown here. These include the following:- (a,d) stained with toluidine blue to demonstrate the anatomy; (b) demonstrating the distribution of α_2 adrenoceptors; (c) control; (e) demonstrating the distribution of α_1 adrenoceptors; (f) demonstrating the distribution of muscarinic cholinoceptors.

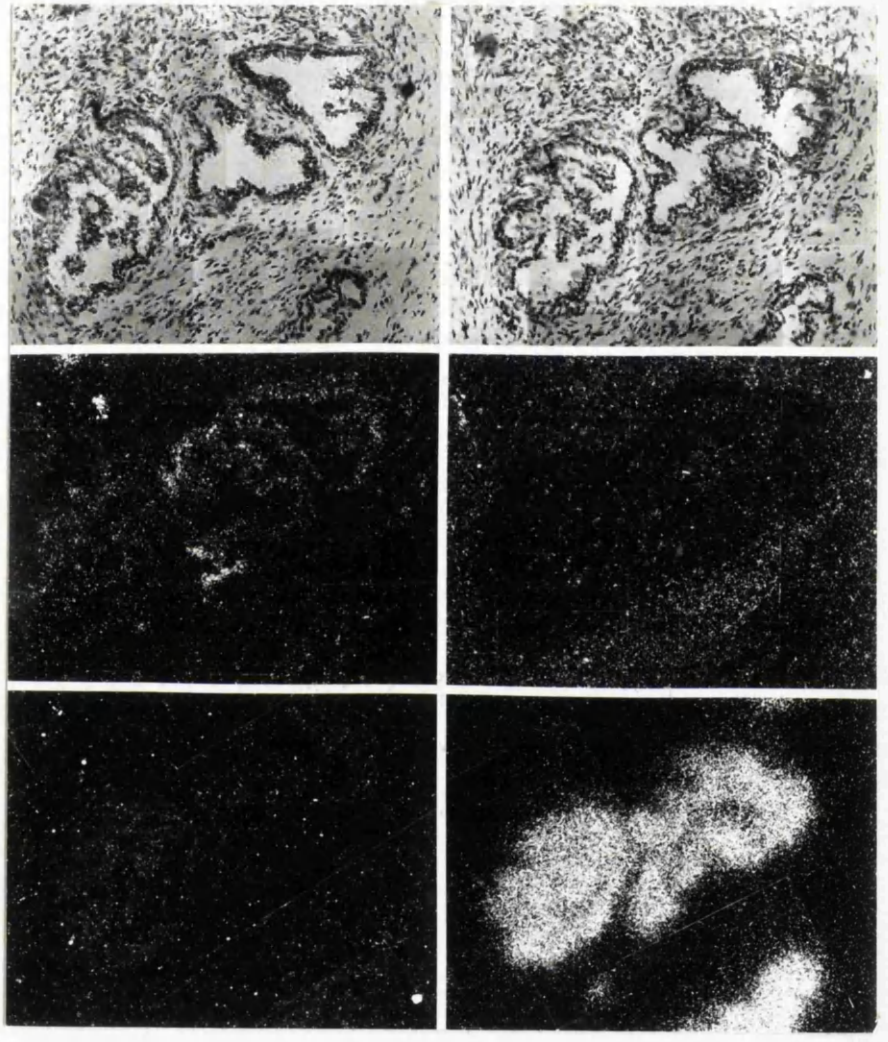


Figure 10.2

A comparison of the binding of ^{3}H-prazosin and ^{3}H-rauwolscine to the stroma of human prostate removed for BPH. (a-f) represents the same area from consecutive sections of the same specimen. (a) and (d) are phase-contrast micrographs of prostatic stromal regions which have been incubated with ^{3}H-rauwolscine (b) and ^{3}H-prazosin (e) respectively. Grain distribution over the stroma (S) is far more dense after incubation with ^{3}H-prazosin (e). (c) and (f) are control sections incubated with ^{3}H-rauwolscine and ^{3}H-prazosin respectively, in the presence of 10^{-5} M phentolamine. Bar = 100 µm.

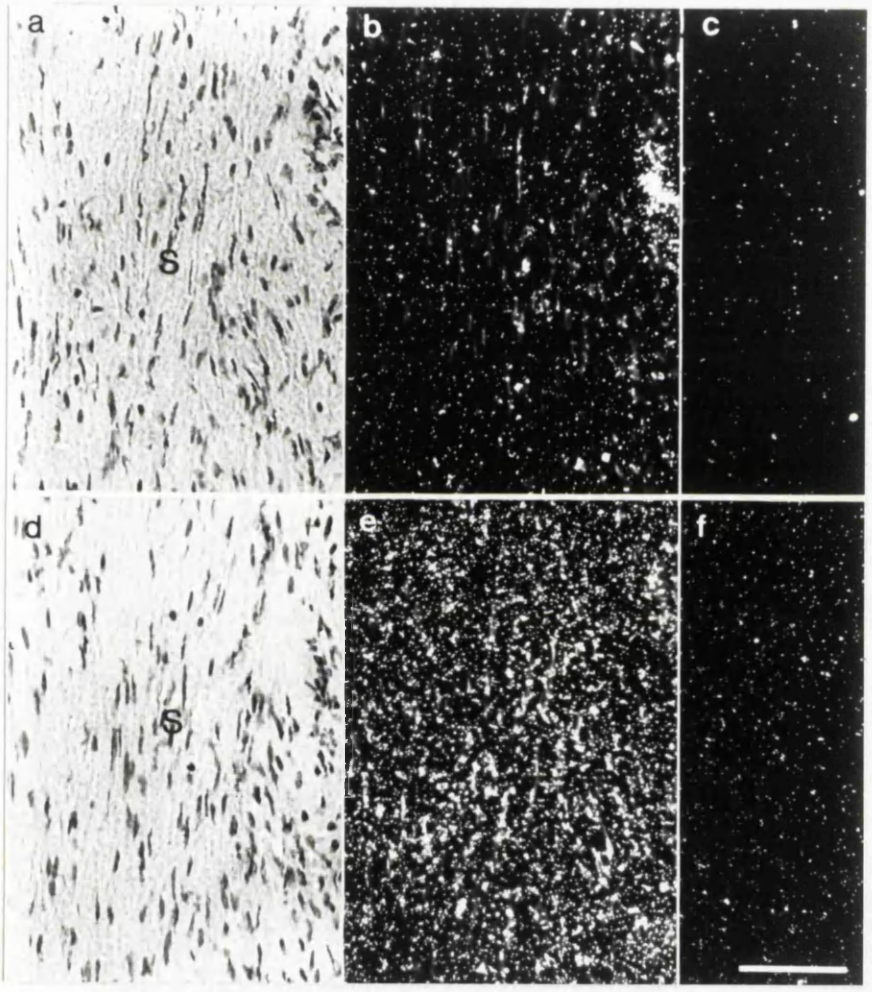


Figure 10.3

Comparison of the binding of radioligands over different areas of human adenomatous prostatic tissue. (a,b) and (d,e) represent the phase-contrast and dark-field views of the same section, whilst (c) and (f) are dark-field views of the consecutive section. Phase contrast micrograph (a) shows glandular epithelium (E) adjacent to prostatic stroma (S). In (b), the majority of binding, after incubation with the α_1-adrenoceptor ligand, ^{3}H-prazosin, is on the stroma. (S), with no specific epithelial (E) binding. L=lumen. In comparison, the binding of the muscarinic cholinoceptor ligand, ^{3}H-QNB (c) is localised to the epithelium (E) with little present in the stroma (S) or lumen (L). A blood vessel (bv), situated in the prostatic stroma (S) is shown in the phase contrast micrograph (d). (e) illustrates the dark field view after binding with ^{3}H-prazosin. Note the sparse binding to the blood vessel (bv), and the increasing grain density over the stroma (S) away from the blood vessel. In contrast (f) is a dark field view of a consecutive section after binding with the α_2 adrenoceptor ligand, ^{3}H-rauwolscine. Note the highly localised binding to the blood vessel (bv) in contrast to the sparse stromal binding (S). Bars = 50 µm.

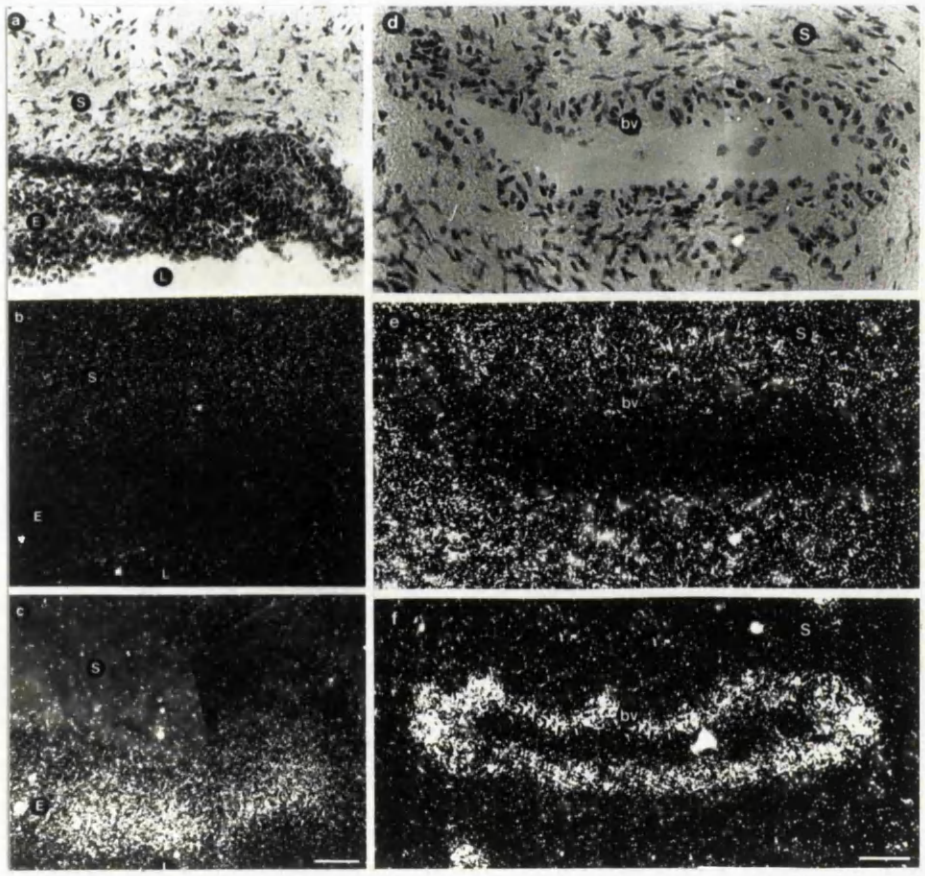


Figure 10.4

Localization of ^{3}H-rauwolscine binding sites to slide mounted tissue sections of adenomatous prostatic tissue. (a) is a phase-contrast micrograph showing glandular epithelium (E) and stroma (S). Several specific areas in close proximity to the base of the epithelial cells have a high α_2 adrenoceptor concentration (*). These areas of high grain densities (*) can be seen in the corresponding dark field photomicrograph (b). (c) is a dark field view of a consecutive control section incubated in the presence of 10^{-5} M phentolamine. Bar = 100 µm.

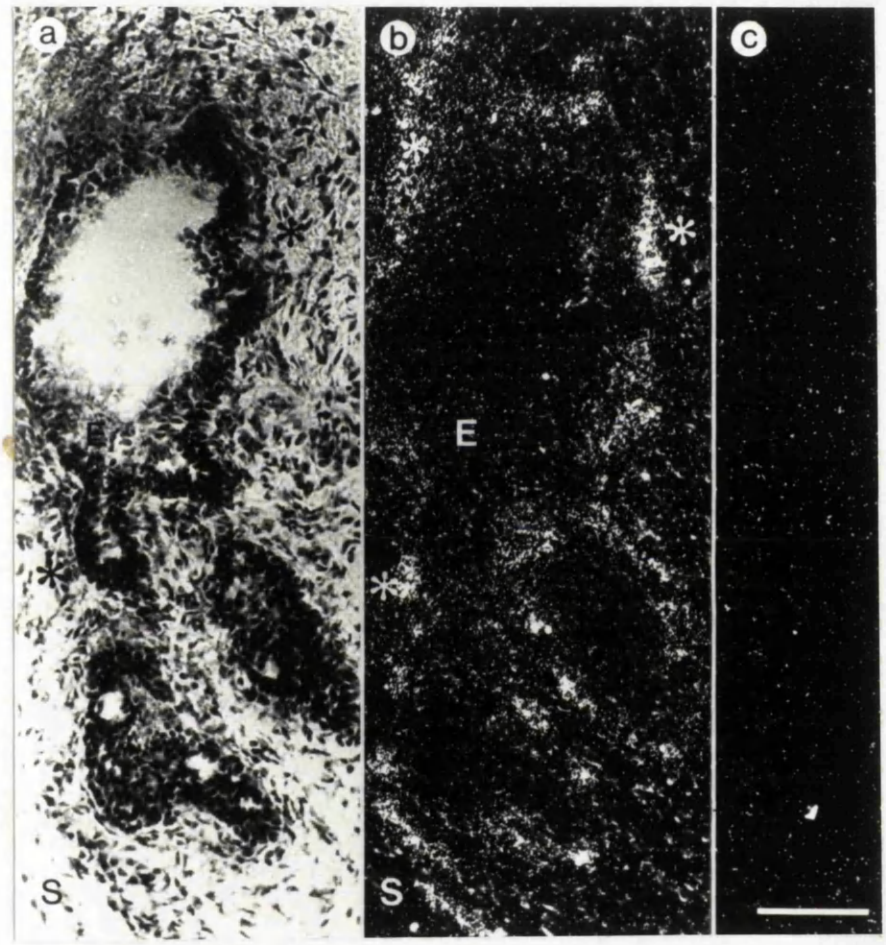
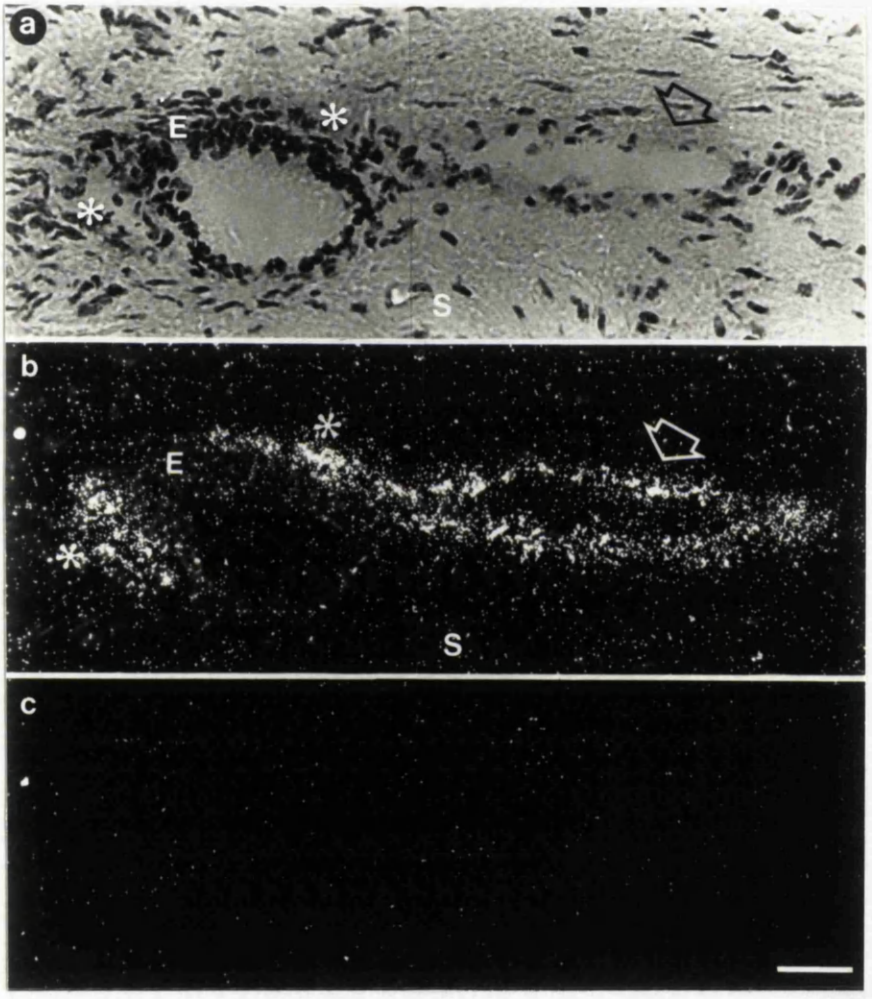


Figure 10.5

Autoradiographical localisation of 3H-rauwolscine binding to a tissue section of hyperplastic human prostate removed for BPH. (a) is a phase-contrast micrograph of the tissue section showing epithelium (E), stroma (S) and a blood vessel (arrow). (b) shows a dark field photomicrograph of the same section. Dense labelling over the blood vessel is seen (arrow). Specific areas in close proximity to the glandular epithelium (E) are also labelled (*). A sparse distribution of grains is seen over the stroma (S). (c) non-specific binding to a control consecutive tissue section (defined by 10^{-5} M phentolamine). Bar = 50 μm.



10.4 DISCUSSION

From the results presented in the previous chapter it is evident that anatomical, functional isometric, and ligand-binding studies agree in suggesting that contraction of prostatic smooth muscle occurs in response to sympathetic stimulation acting principally via α_1 receptors. Confirmatory proof for an association of this type however requires the specific localisation of binding sites by autoradiographic techniques.

The specific localisation of neural receptor binding sites within human prostate has only previously been reported for muscarinic cholinoceptors (Lepor and Kuhar, 1984; Hedlund et al. 1985). These receptors are located predominantly in the epithelium of glandular acini at a concentration seventy-fold higher than in the prostatic stroma, a finding confirmed by these experimental results. This study presents the visual localization of α_1 and α_2 adrenoceptors in the human prostate, by an in vitro autoradiographic technique. Receptor autoradiography has two substantial advantages over biochemical studies, in that it combines specific anatomical resolution with high sensitivity. The availability of slide-mounted intact histological sections allowed densitometric computer-assisted quantification of the α-adrenoceptor binding within prostatic stromal tissue. This degree of specificity is not achieved in homogenate studies. A discrepancy exists in the literature as regards the adrenoceptor density in human prostatic tissue (Hedlund et al. 1985, Shapiro and Lepor 1986), but the present study clearly shows an α_1 adrenoceptor predominance over α_2 adrenoceptors in the prostatic stroma;

which is consistent with the pharmacological data (Chapter 9). The three receptors studied here are principally situated in different prostatic compartments (Table 10.2), and the maps of receptor distribution produced may be useful in predicting response to drug treatment.

Historically, the pharmacological and functional dissimilarity between pre-junctional α-adrenoceptors on post-ganglionic sympathetic neurons and post-junctional α-adrenoceptors on effector cells led to the designation α_1 for post- and α_2 for pre-junctional adrenoceptors (Langer 1974). However, more recent work suggests the existence of post-junctional α_2 adrenoceptors in some smooth muscle (Timmermans and Van Zwieten 1981, McGrath 1982). Since in both normal and hyperplastic prostate there is a functional predominance of α_1 adrenoceptors, the role of the population of α_2 adrenoceptors in prostatic tissue is at present unresolved, although they may be largely concerned with pre-junctional modulation of transmitter release. Whereas a sparse α_2 adrenoceptor population was associated with the prostatic stroma, dense labelling was seen on blood vessels. The possibility that there might be two populations of vascular α-adrenoceptors, only one of which was susceptible to blockade with prazosin, was first suggested by Moulds and Jauernig (1977) and Jauernig, Moulds and Shaw (1978) in isolated strips of human palmar digital arteries. Subsequently, post-junctional α_2 adrenoceptors have been identified in various vascular beds (DeMay and Vanhoutte 1980, Kiowski et al. 1983, Van Brummelen et al. 1982 and Hirst and Neild 1980). Although receptors visualised in this study

could be pre-junctional in close association with perivascular adrenergic nerves, the possibility that both α_1 and α_2 adrenoceptors mediate smooth muscle contraction, and in particular vasoconstriction, could have important therapeutic implications.

Muscarinic cholinergic receptors have been found exclusively on glandular epithelium in this and other reports. Both adrenergic and cholinergic nerve axons have been detected subepithelially in the human prostate (Vaalasti and Hervonen 1980a), and cholinoceptor stimulation, resulting from sympathetic activity in the canine prostate, causes an increase in the amount of secretion expelled into the urethra (Bruschini et al. 1978). In this study, occasional high α_2 receptor densities were found in close proximity to these epithelial cells bearing muscarinic receptors, in the region of the basal lamina. This arrangement is a well-recognized feature in the autonomic nervous system. For example, α_2 adrenoceptors have been reported on cholinergic nerve terminals in the guinea-pig ileum (Drew 1978). It is interesting to speculate that in the prostate gland there could be adrenoceptor modulation of acetylcholine release.

This study has enabled the localisation of receptors to different compartments of the human prostate gland. Although the evidence suggests that the α_1 adrenoceptor is of primary importance in the mediation of muscular contraction, and conversely, the muscarinic cholinoceptors are related predominantly to glandular function, the role of prostatic α_2 adrenoceptors is still a matter of speculation. As it has been suggested that

combined α_1 and α_2 blockage, such as with the drug phenoxybenzamine, is superior to the specific α_1 antagonist action of prazosin in the clinical management of prostatic obstruction (Hedlund et al. 1983), it is possible that the α_2 adrenoceptors are not only important in vascular control, but may also have a neuromodulatory function in muscular contraction. However, it seems unlikely that post-junctional α_2 adrenoceptors contribute significantly to outflow resistance. The data presented here support the suggested pre-junctional localisation of α_2 adrenoceptors, where they may be involved in the modulation of transmitter noradrenaline release. Indeed, the use of phenoxybenzamine is associated with a high incidence of side-effects attributed to a blockade of this neuromodulatory pathway, leading to high circulating levels of noradrenaline. Recent work has suggested that α blockade may also act directly on the detrusor muscle (see Chapter 5); nevertheless, the specific receptor populations involved in the obstructed bladder still need to be defined.

This study has clarified the regional distribution of α adrenoceptors in adenomatous prostatic tissue and provides clear ultrastructural evidence to support the observed action of α blockade of the human lower urinary tract both in in vitro studies and clinical trials. No comparable study has been performed on the non-hyperplastic or capsular prostatic tissue. Such a study would be of considerable interest, not only to increase understanding of the sympathetic nervous control of normal prostatic tissue, but also, in view of the suggested differences in embryological origin (Hutch and Rambo 1970)

and distinct pharmacological responses (Caine et al. 1975), to distinguish individual regions of the prostate.

CHAPTER 11

ANATOMY AND INNERVATION OF THE MALE BLADDER NECK

11.1 REVIEW OF THE LITERATURE.

The precise mechanism of the closure of the bladder neck and its opening during voiding is not known and has been subject to much debate. Galen described a distinct bundle of muscle fibres encircling the bladder neck (Denos 1914) a view supported by a number of other investigators (Kohlrausch 1854, Learmonth 1931). These observations equated well with the simple functional hypothesis that there was a reciprocal innervation whereby, when the bladder contracted under parasympathetic stimulation, then the sympathetically innervated bladder neck relaxed.

Other workers have challenged this view on structural grounds (Griffiths 1891, Young and Wesson, 1921). In particular, Wesson (1920) proposed the concept of a sphincteric action in the absence of a sphincteric muscle; with opposing inner and outer loops arising from the bladder muscle and acting on the bladder neck, which are subsequently pulled open by contraction of the trigonal muscle. Anatomical studies from McCrae (1926) are basically in agreement with Wesson who suggested that striated muscle from the distal sphincter muscle ascends to the level of the bladder neck where it fuses with the outer loop to surround the smooth muscular internal sphincter and aid opening of the bladder neck.

Tanagho and Smith (1966) in a comprehensive anatomical and histological study of 24 human bladder necks concluded that there was no

evidence of an anatomical sphincteric mechanism at the bladder neck. They reported that the outer circular layer of the bladder formed the outer coat of the proximal urethra and provided a passive occlusive effect at the bladder neck. There was a ventral condensation of the middle circular layer of the detrusor, which they suggested could be important in producing funnelling of the bladder outlet during voiding; the superficial trigone then acting in conjunction with the inner longitudinal coat of the bladder to help to open the bladder neck. The involvement of the sympathetically innervated trigone was advanced to explain the sympathetic innervation of the bladder neck (Learmonth 1931).

The first definitive proof that there was a distinct anatomical and functional sphincteric mechanism of the male bladder neck (Turner-Warwick 1971) followed on from the perfection of video-urodynamics as a clinical technique (Bates 1970). Anatomical studies reported by Gosling (1979, 1983) and Dixon and Gosling (1987) supported the view that the male bladder neck comprises a collar of smooth muscle cells forming a separate functional unit from the bladder. Study of the bladder neck smooth muscle reveals that it is histologically, histochemically and pharmacologically distinct from that which comprises the detrusor proper (Nergardh and Boreus 1972, Kluck 1980, Gosling Dixon et al. 1983).

The current consensus is that the bladder neck is only well developed as a sphincteric mechanism in the male, serving a genital function in relation to ejaculation. In the absence of a distal sphincter mechanism (eg:

following injury or prior surgery), the bladder neck mechanism is sufficiently powerful to maintain continence. Detailed anatomical studies reveal two layers, a powerful inner muscular structure containing a rich sympathetic nerve supply, contraction of which prevents retrograde ejaculation and an outer urinary sphincter with predominantly parasympathetic innervation (Dixon and Gosling 1987). Distally the bladder neck muscle is continuous with the pre-prostatic sphincter which merges with and becomes indistinguishable from the musculature, the stroma and capsule of the prostate gland.

In contrast it is now well recognised that in the asymptomatic nulliparous woman the bladder neck is often open when assessed by ultrasound examination and is therefore by inference functionally incompetent (Chapple et al. 1989b). Not surprisingly therefore, the arrangement of smooth muscle in this region is entirely different in males and females (Gosling et al.1977). Furthermore, morphological studies have failed to demonstrate a significant population of catecholamine-containing fibres innervating the female bladder neck (Gosling 1986).

The male bladder neck receives both a parasympathetic and a sympathetic innervation (Gosling and Thompson 1977), but it seems likely that contractile responses are predominantly mediated via the sympathetic nervous system (Learmonth 1931, Stockamp and Schreiter 1974, Walker and Bates 1985). Excitatory α-adrenergic receptors can be identified in the bladder outlet region (Edvardsen and Setekleiv 1968, Nergardh and Boreus

1972, Awad et al. 1974).

There is clear evidence of extensive neuronal interaction at the bladder neck. Histological studies of the intramural innervation of the bladder and urethra in the cat (Hamberger and Norberg 1965, Elbadawi and Schenk 1966) have reported the presence of adrenergic connections with cholinergic ganglia. In vitro pharmacological studies in the cat have shown that parasympathetic stimulation with acetylcholine stimulates short sympathetic neurons resulting in the release of noradrenaline (Nergardh and Boreus 1973); an alternative mechanism is that acetylcholine stimulates calcium uptake at adrenergic nerve terminals, prompting the subsequent release of noradrenaline. In a study of the dog bladder neck Mutoh and colleagues (1987) provided clear evidence that muscarinic receptors on peripheral sympathetic nerve terminals were able to alter nerve-mediated electrical stimulation of tritiated noradrenaline release.

No comprehensive studies of neuropeptide localisation within the bladder neck have previously been reported. Although Alm et al. (1980) studied the distribution of VIP in the genito-urinary tract of various mammals, and reported a rich supply of VIP nerves within the urethra and prostate and identified the presence of numerous associated VIP-positive ganglia, they did not specifically comment on the innervation of the bladder neck. Gu et al. (1984) carried out a comprehensive study of the distribution of the neuropeptides VIP, substance P, somatostatin and NPY within the human bladder and trigone, and similarly failed to comment on the bladder neck.

The mechanism of bladder neck opening is unclear, with debate as to whether it is an active process or a passive phenomenon resulting from the inhibition of normal resting sympathetic tone. Little in vivo work has been carried out and in vitro studies provide contradictory results. Certainly neurogenic relaxation has been demonstrated in vitro in both laboratory animals and a few human tissues (Andersson et al. 1983, Klarskov et al. 1987), but this finding has been challenged by other workers (Walker and Bates (1985).

A non-adrenergic non-cholinergic mechanism of action has been postulated for active bladder neck relaxation (Hills et al. 1984) with both 5-HT and VIP satisfying some of the criteria of a neurotransmitter. In other studies of human and porcine bladder neck, nerve-mediated stimulation resulted in TTX-sensitive relaxation which was not abolished by blockade with atropine, phentolamine or propanolol (Klarskov et al. 1983). In vitro experiments on porcine bladder neck have concluded that 5-HT had a potential role in producing active relaxation (Klarskov and Horby-Peterson 1986). In addition, the pig bladder neck exhibits a pronounced relaxation to VIP in vitro, which is slower than the response to nerve-mediated stimulation and not blocked by anti-VIP; a similar but less marked response is also produced by ATP and 5-HT (Klarskov 1987). It must however be concluded from these studies that none of these agents fulfilled any of the criteria of a classical neurotransmitter. It is of particular interest that a combined in vitro histochemical and in vivo study in human volunteers failed

to demonstrate either a correlation between VIP concentrations in bladder neck smooth muscle and bladder outlet pathology or any influence of VIP on urodynamic pressures when infused intravenously into human volunteers (Klarskov 1984b, 1987c).

At present, the physiological and pathophysiological significance of a non-cholinergic, non-adrenergic inhibitory nerve response remains unknown. A possible explanation for the contradictory results that have been reported is that not only is the ability of nerve stimulation to evoke relaxation short lived (as might be expected from a peptide neurotransmitter), but also it only appears to be a significant feature at frequencies less than 10Hz (Klarskov et al.1983); a characteristic observed for NANC innervation in other tissues (Kennedy et al. 1986). Similar conclusions were reached by Speakman et al. (1988) from their study of NANC transmission in the human trigone.

The prostate and bladder neck are likely to exhibit a number of structural and functional similarities; nevertheless the bladder neck is predominantly of importance as a sphincteric mechanism, whilst the prostate is primarily a glandular structure, subserving a secretory function.

Whilst it is now clearly established that the bladder neck receives its most important innervation from the sympathetic nervous system via adrenergic nerves, the contribution provided by other nerve types has not previously been studied in man. The following section of this work describes a study of the innervation of the male bladder neck by AChE-positive and a number of NANC neurons.

11.2 HISTOCHEMICAL STUDIES

Tissues were obtained from 36 patients with full informed consent. All of these patients were already included in the co-existing histochemical and pharmacological studies of prostate and bladder reported elsewhere in this thesis. Bladder neck tissue was obtained at the time of surgery and fixed to a small piece of cork using the tissue mounting solution "Tissuetek". The tissue was then subdivided into two portions.

a) One portion was immediately frozen in theatre in isopentane (2-methylbutane) which had been previously cooled directly in liquid nitrogen to -160 °C. The frozen tissue was then carefully labelled and preserved in a store at -70 °C. The frozen tissue was transported to laboratories either packed around with dry ice or in a container immersed in liquid nitrogen and again stored at -70 °C. In the laboratory, the material was removed from the -70 °C store and allowed to equilibrate for 5 minutes in a -20 °C cryostat. Serial sections were cut from each tissue block with a microtome at thicknesses of 10-20 μm. The remainder of this frozen tissue was submitted to biochemical assay.

b) The other portion of tissue was submitted for immunofluorescence studies; tissue was fixed rapidly in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS), pH 7.10-7.4 for 2 h at 4 °C, rinsed in PBS containing 7% sucrose and 0.01% sodium azide, and left overnight at 4 °C in the same buffer.

A potential criticism of the material obtained in this study, since it was

obtained at the time of prostatectomy, is the difficulty of ensuring that it was purely bladder neck without a significant component being prostatic tissue. All of the material submitted to neural quantification was screened histologically and any evidence of prostatic acini within the tissue invalidated it from further study.

11.2.1 PATIENTS

Histochemical studies

Bladder neck tissue was obtained from 2 patients, with no evidence of bladder outflow obstruction, who were undergoing cystectomy for carcinoma and who had not received pre-operative radiotherapy (mean age 72.0 ± 1 year). In a further 34 patients, bladder neck tissue was removed at the time of prostatectomy; all of these patients had been categorized preoperatively on the basis of clinical presentation and full urodynamic evaluation using video cystometrography. The composition of the study population was as follows:- unstable obstruction n=15 (mean age 67.5 ± 1.98 years), stable obstruction n=7 (mean age 65.14 ± 2.46 years), acute retention n=6 (mean age 17 ± 2.01 years), chronic retention n=3 (mean age 65.33 ± 4.81 years), and post-TURP n=3 (mean age 71.33 ± 5.84 years). AChE staining was carried out in all of these patients; in only 23 patients was sufficient tissue available to allow parallel study of dopamine β hydroxylase (DBH)-, vasoactive intestinal polypeptide (VIP)- and neuropeptide Y (NPY)-immuno-reactivity. This second study population included the following groups; stable obstruction n=7 (mean age 65.29 ± 1.76 years), unstable obstruction n=10

(mean age 66.89 ± 2.78 years) and acute retention of urine n=6 (mean age 70.83 ± 3.19 years). The individual groups detailed here were age-matched, with no significant differences evident at statistical comparison. The difficulty experienced in obtaining control bladder neck tissues was highlighted by the small number of patients who could be studied within this category.

Biochemical assay

Histochemical assay of neurotransmitter content in bladder neck tissue was only possible in a proportion of patients in each of the study groups because of the shortage of material. The groups comprised the following; stable obstruction n=5 (mean age 69 ± 1.85 years), control n=4 (mean age 68.3 ± 2.1 years), unstable obstruction n=10 (mean age 66.89 ± 2.78 years), acute retention n=6 (mean age 70.83 ± 3.19 years).

11.2.2 METHODS

HISTOLOGICAL TECHNIQUES

A. Routine Histology and AChE staining.

Masson's (1929) trichrome technique was employed for routine histological purposes. AChE was localised according to the method of Gomori (1952).

B. Immunohistochemistry

The tissue sections (10 µm) were cut on a cryostat and immuno-fluorescence staining was carried out using the indirect method (Coons et al. 1955). (For further details see Chapter 5).

C. Quantitative Methods for Light Microscopy

Analysis of neural imaging using AChE-positive staining and immunofluorescence studies was carried out in a blinded fashion using both quantitative and semiquantitative techniques (see chapter 5 for further details).

NEUROCHEMISTRY

Peptide assay

Segments of tissue were stored as detailed above in liquid nitrogen until peptide extraction. The bladder tissue was weighed and the peptides extracted into 0.5 M acetic acid in polypropylene tubes in a boiling water bath for 15 min. The samples were homogenized, centrifuged for 30 min at 3500 g and lyophilized. (For further details see Chapter 5).

11.2.3 STATISTICAL ANALYSIS

Statistical analyses were performed to contrast patients with controls and compare results obtained in each sub-group where appropriate. Although much of the data approximated to a normal distribution, many groups studied contained small patient numbers and statistical analysis was therefore carried out treating the data as though it were non-parametric; the Mann Whitney U-test being used to test for statistical significance. Data analyses were carried out with the aid of the Minitab 5.1.1 software package on a Tandon IBM compatible computer.

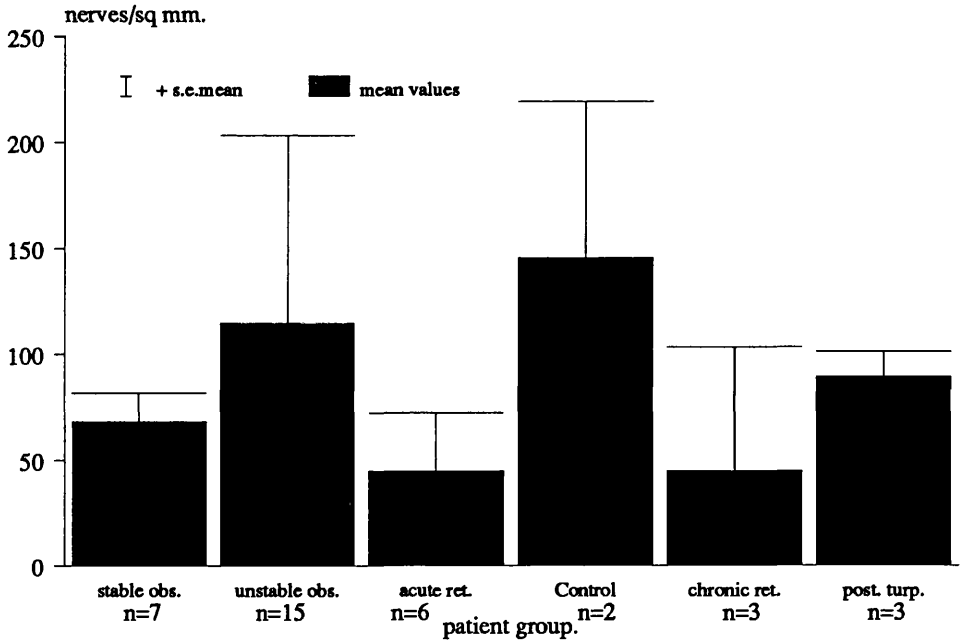
11.3.RESULTS

11.3.1 Neural Quantification.

Although a dense innervation with AChE stained nerves was evident (Figure 11.1), the density of DBH-immunoreactivity (a non-specific marker for noradrenergic nerves) was between 3- and 15-fold less than this (Figure 11.2a). Objective quantification of immunoreactivity of nerves to a number of putative peptide neurotransmitters revealed no evidence of immuno-reactivity to somatostatin or substance P and only traces of CGRP within the unstable obstructed group (Figures 11.3a, 11.4a, 11.5a). The greatest density of immunoreactivity was to NPY (see figure 11.6a) and VIP (see figure 11.7a). All of the immunoreactive nerves were uniformly distributed within the connective tissue between muscle fibres. Apart from the differences in the results of AChE quantification in the subgroups unstable obstruction and post-TURP ($P < 0.05$), the differences in neural count in each of the population of study did not reach statistical significance. Interestingly, conventional subjective neural quantification was carried out in each group and revealed similar findings to the objective technique (see figures 11.2b-11.7b).

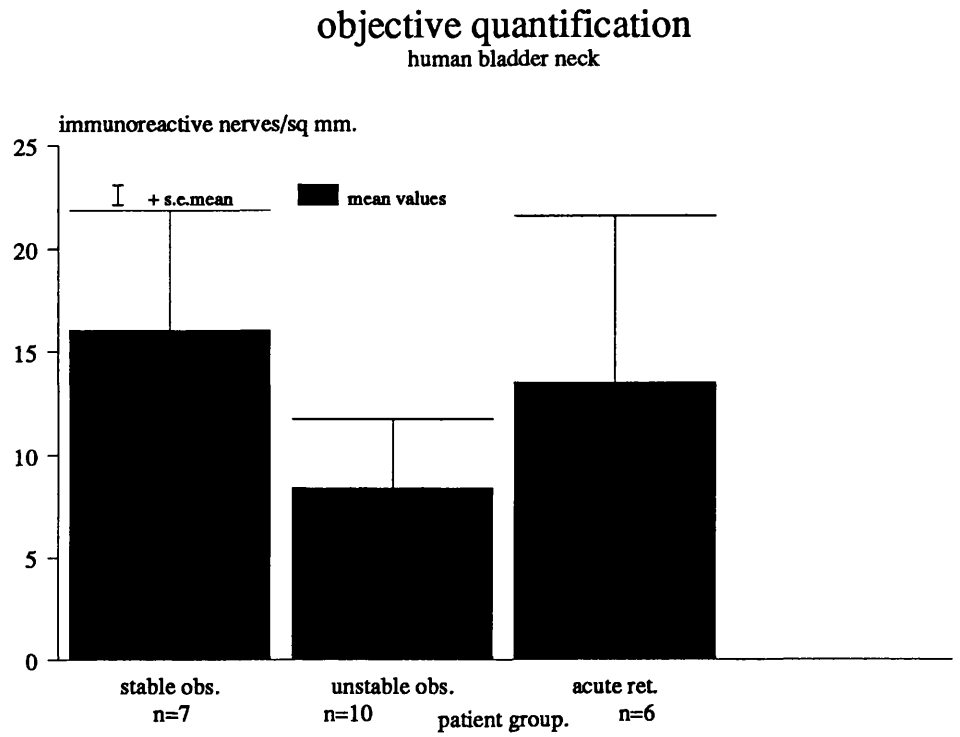
Figure 11.1

objective quantification.
human bladder neck.

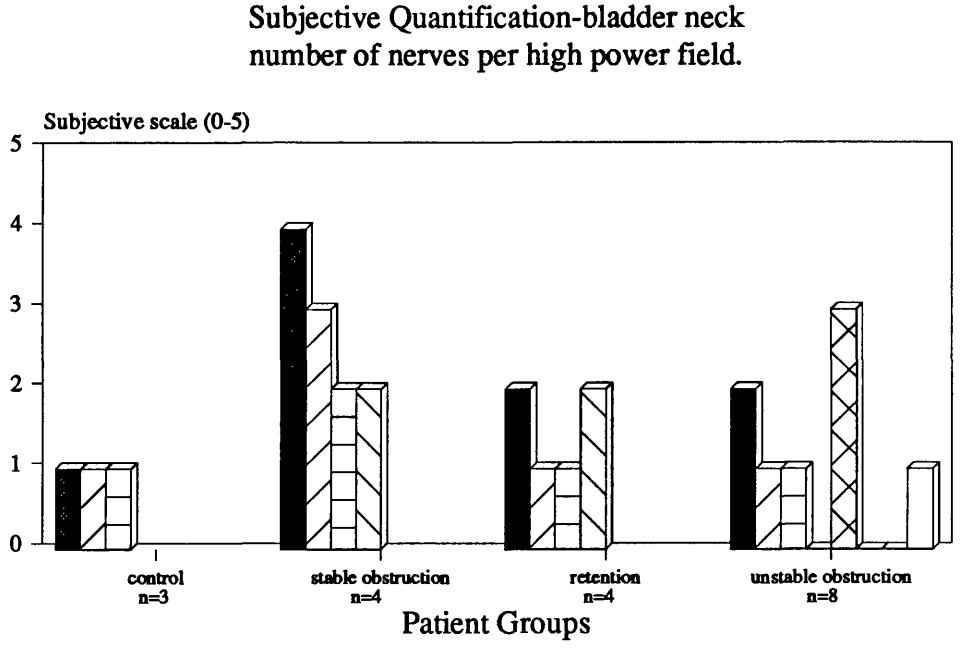


Acetyl-cholinesterase stained nerves.

Figure 11.2

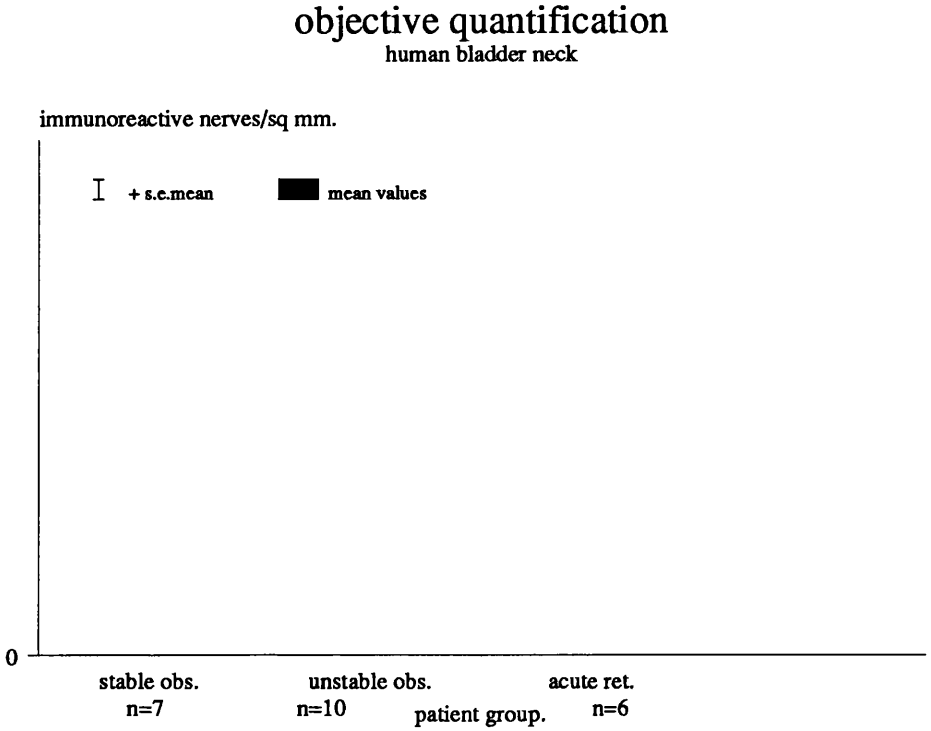


DBH - immunoreactive nerves

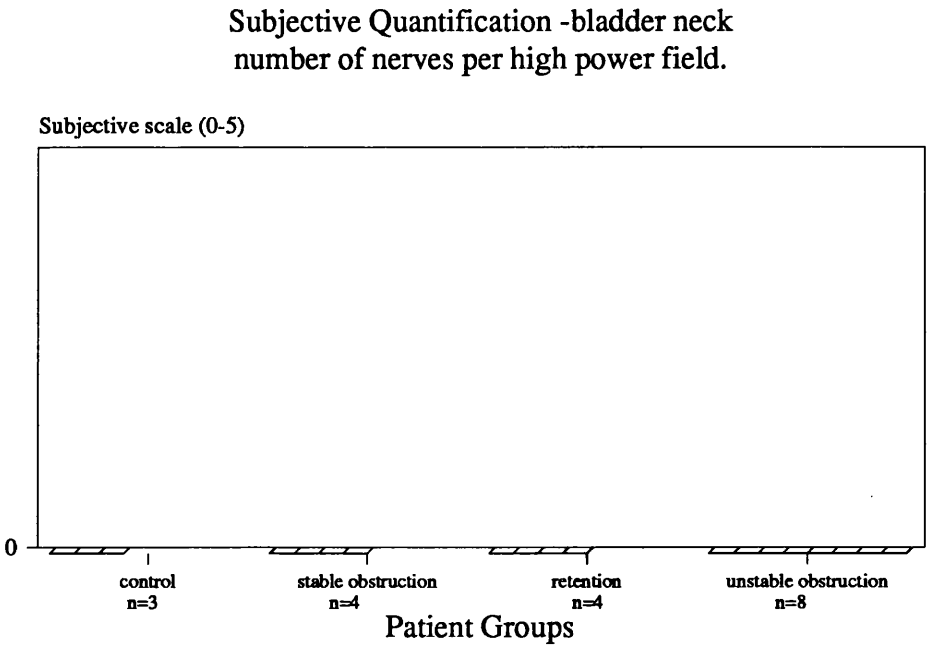


DBH - IMMUNOREACTIVE NERVES

Figure 11.3

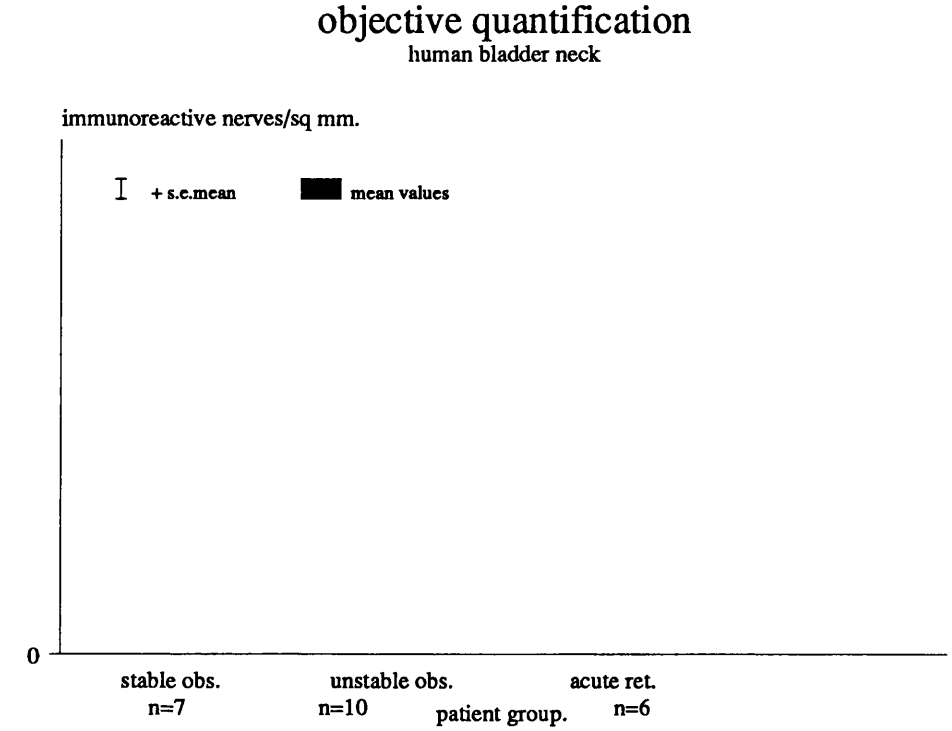


SOM - immunoreactive nerves

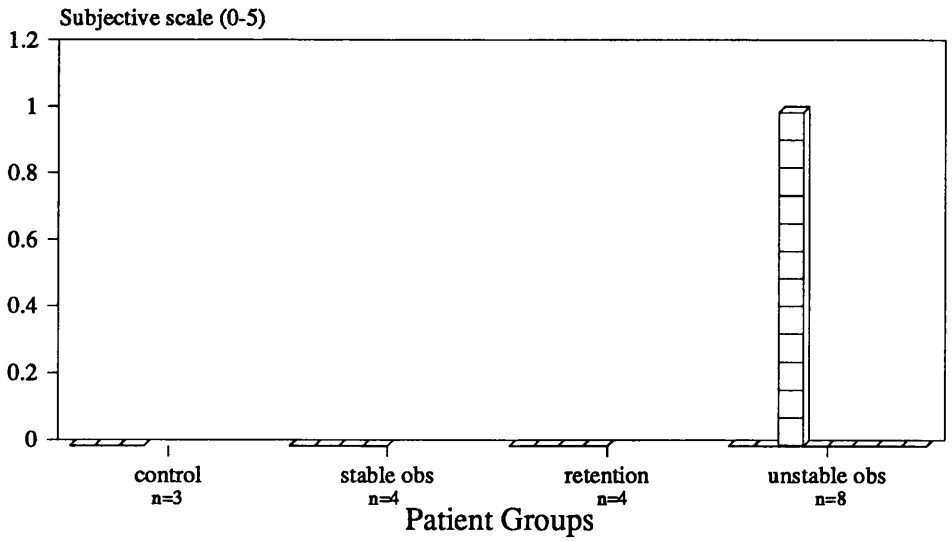


SOM - IMMUNOREACTIVE NERVES

Figure 11.4

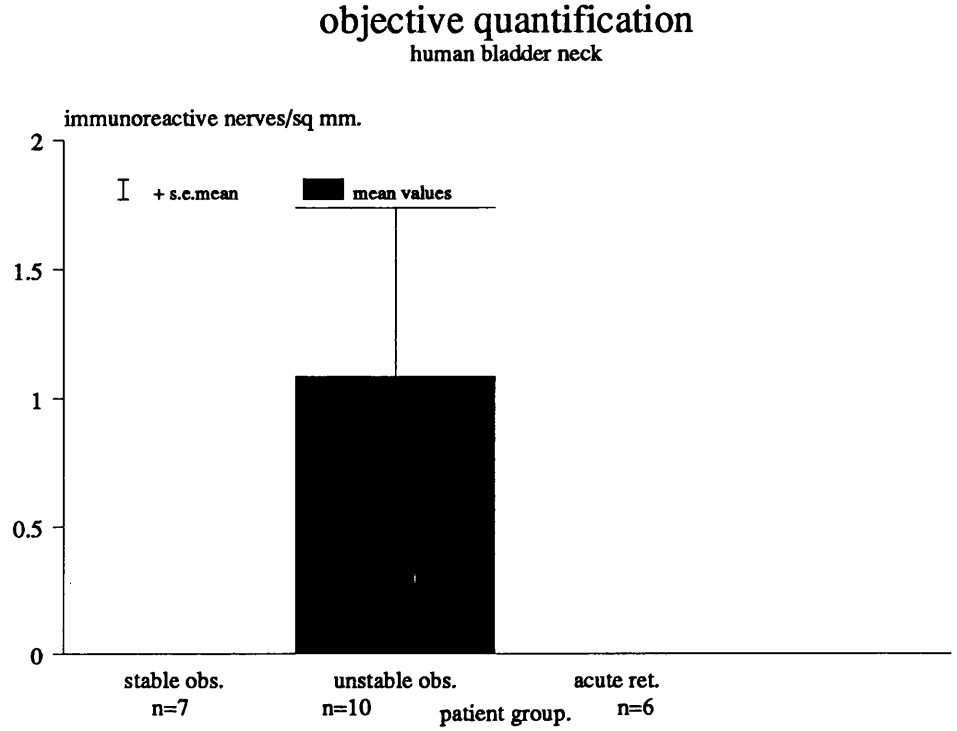


Substance P - immunoreactive nerves

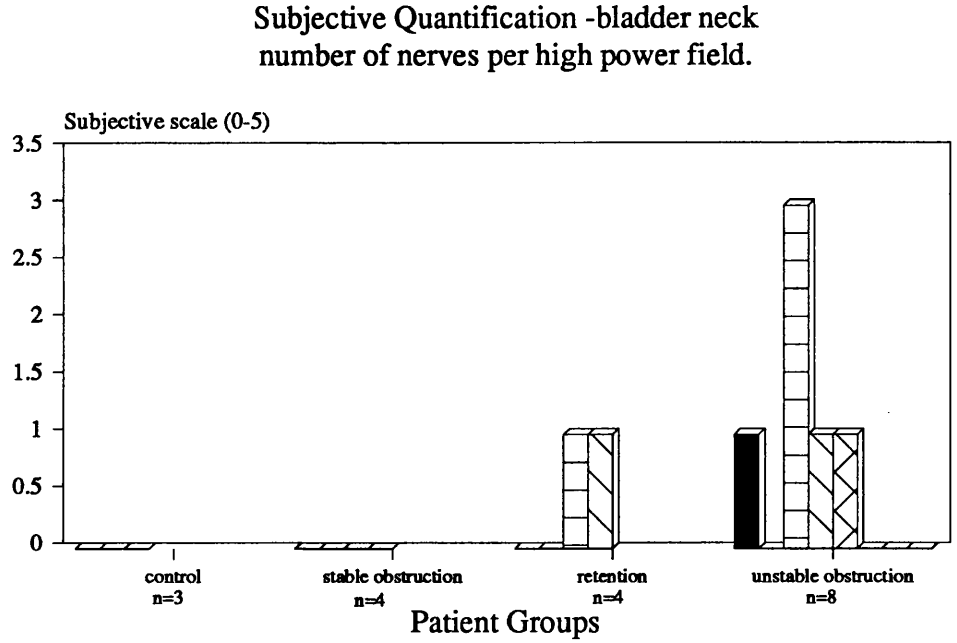


SP - IMMUNOREACTIVE NERVES

Figure 11.5

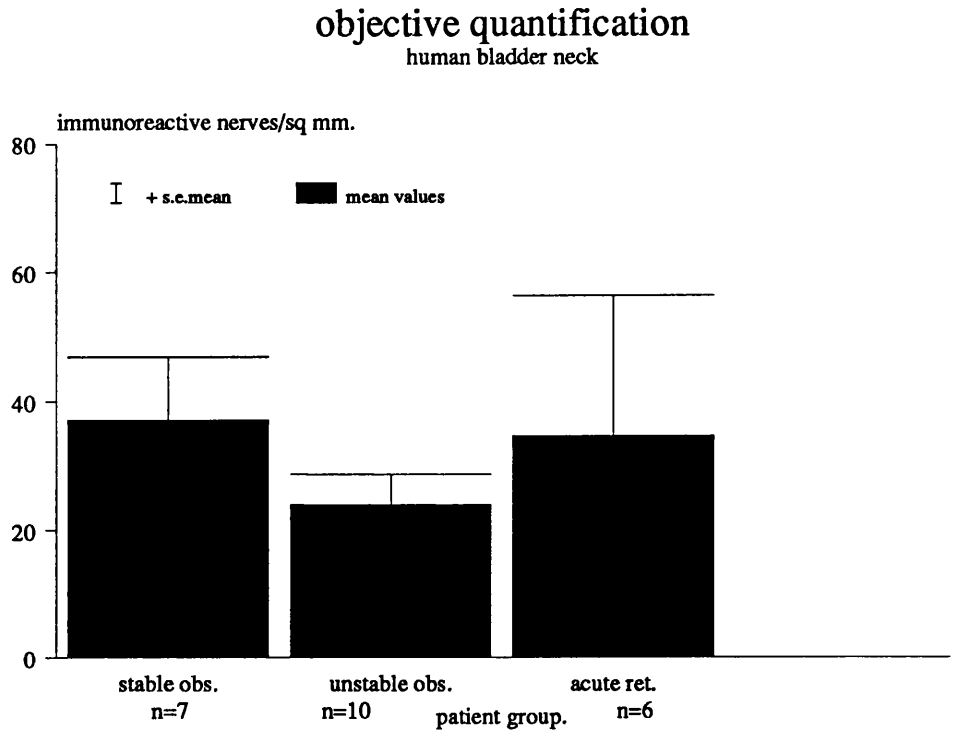


CGRP - immunoreactive nerves

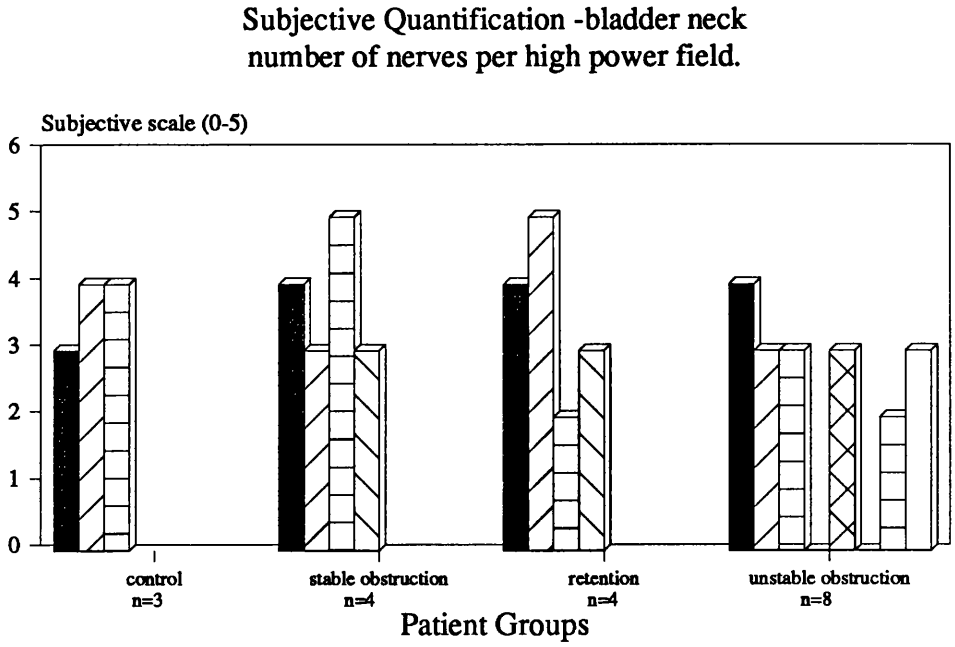


CGRP- IMMUNOREACTIVE NERVES

Figure 11.6

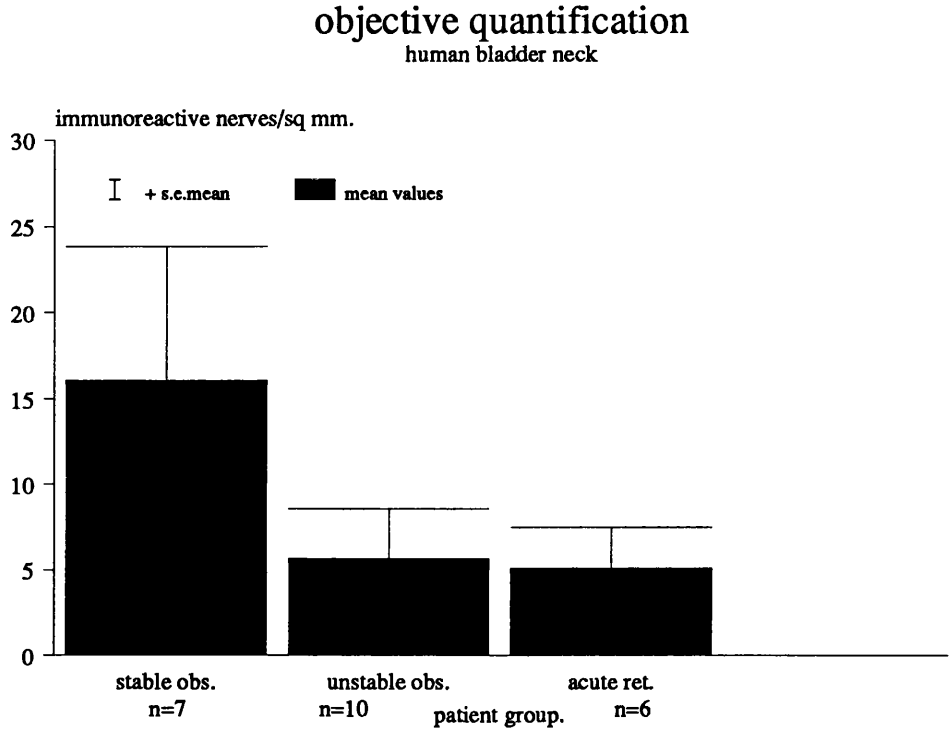


NPY - immunoreactive nerves

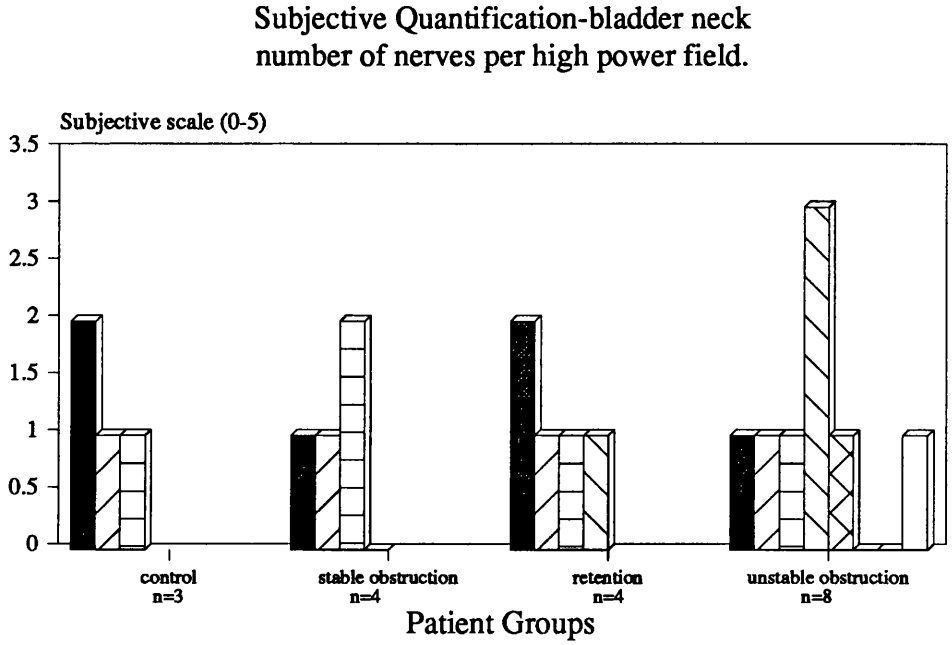


NPY - IMMUNOREACTIVE NERVES

Figure 11.7



VIP - immunoreactive nerves



VIP - IMMUNOREACTIVE NERVES

11.3.2 Biochemical Assay.

Histochemical assay of noradrenaline in the bladder neck revealed a significant increase in its content in tissue from the unstable obstructed group ($P < 0.02$) and the stable obstructed group ($P < 0.05$) as contrasted to control (see figure 11.8). Study of the content of putative sensory peptides CGRP and SP revealed a significant increase in CGRP in the stable obstructed group ($P < 0.05$, Figure 11.9a) and a significant decrease in substance P in the unstable obstructed group ($P < 0.02$) contrasted to control (Figure 11.9b). A similar study of NPY demonstrated no significant differences between the groups (Figure 11.10a) whereas VIP levels were significantly increased in the unstable obstructed group as contrasted to control ($P < 0.05$) (see Figure 11.10b).

Figure 11.8

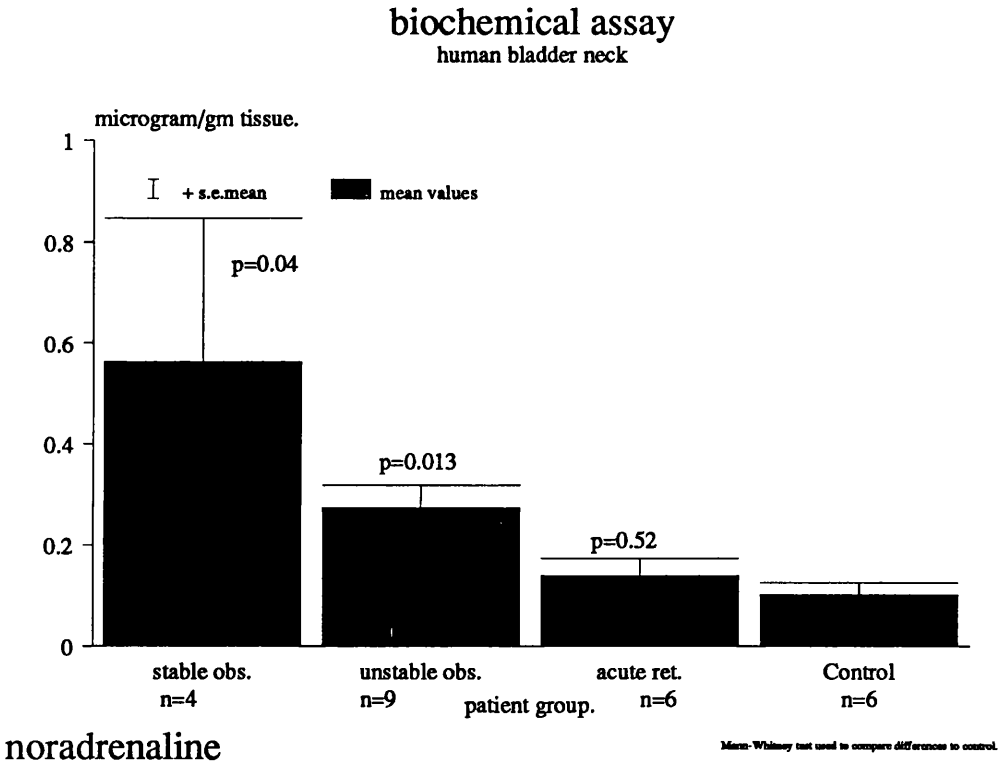
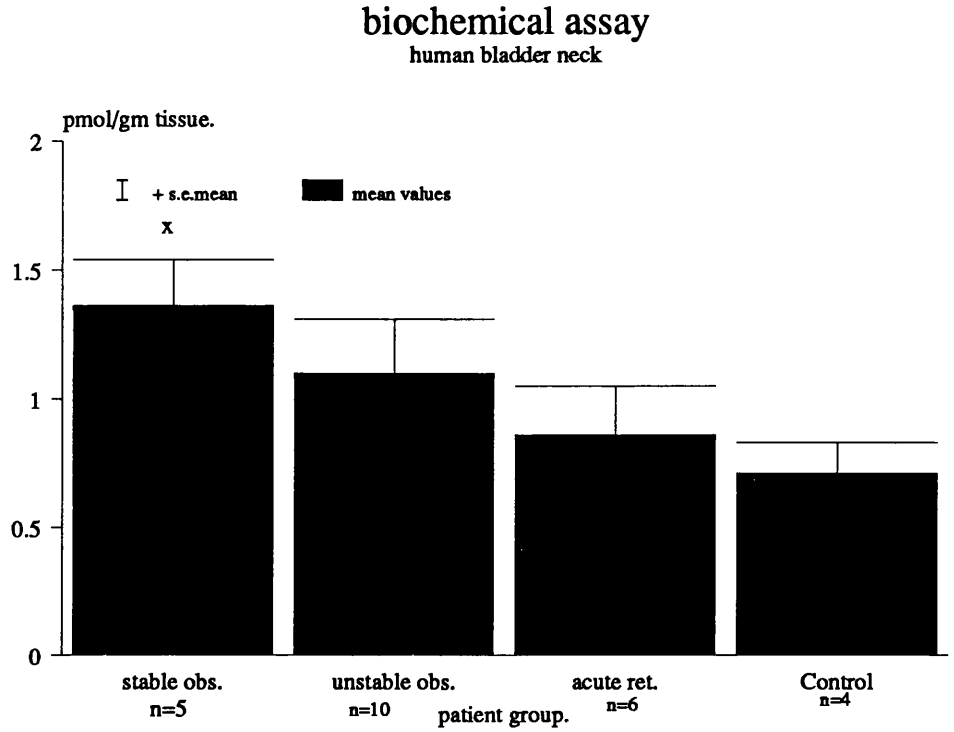
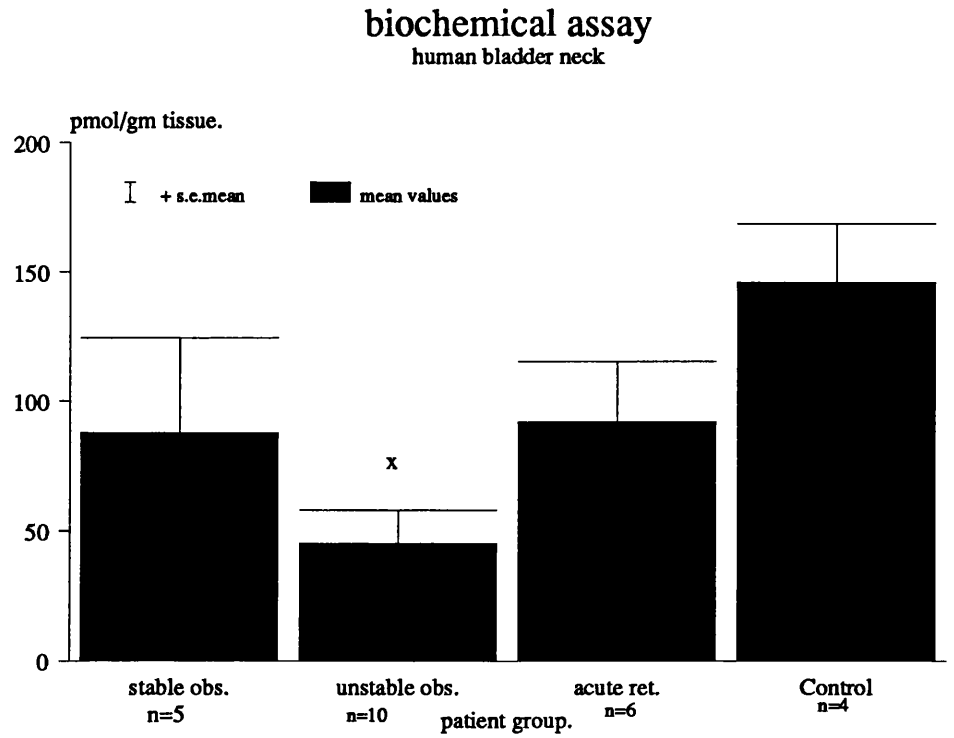

biochemical assay
human bladder neck
microgram/gm tissue.
1
0.8
0.6
0.4
0.2
0
+ s.e.mean
mean values
p=0.04
p=0.013
p=0.52
stable obs.
n=4
unstable obs.
n=9
acute ret.
n=6
Control
n=6
patient group.
noradrenaline
Mann-Whitney test used to compare differences to control.

Figure 11.9



CGRP

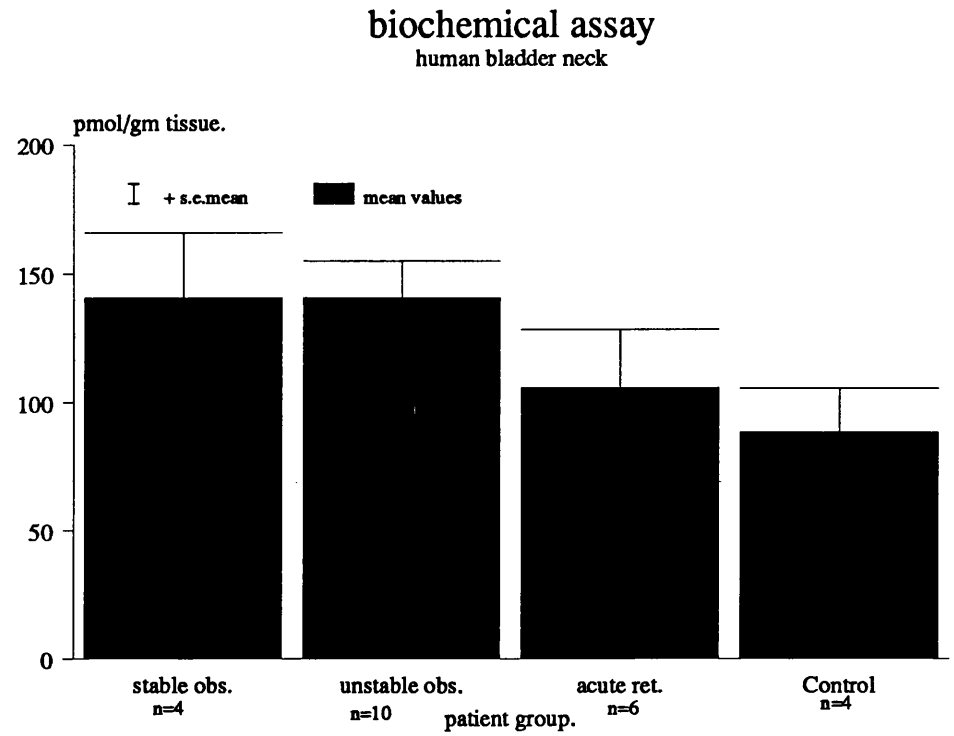
Statistical comparison to control (Mann Whitney $p<0.05$).



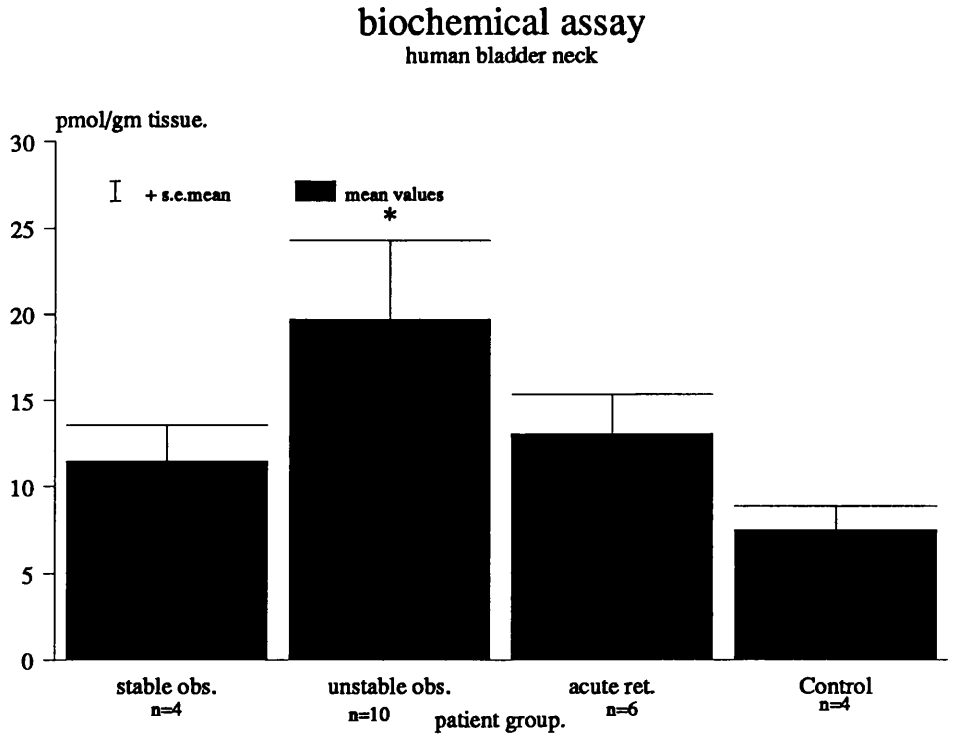
SUBSTANCE P

Statistical comparison to control =x
$p<0.05$(Mann Whitney).

Figure 11.10



NPY



VIP

*=p<0.05 (Mann-Whitney) as compared to control.

11.4 DISCUSSION

Whilst the functional importance of the human male bladder neck is indisputable, review of the world literature reveals a dearth of knowledge relating to its innervation and structure. It is clear that the male human bladder neck contracts primarily under the influence of its sympathetic innervation, but the significance of the dense AChE-stained population as contrasted to the corresponding paucity of DBH-immunoreactivity remains obscure. It must be remembered that acetylcholinesterase (AChE) and dopamine β hydroxylase (DBH) are indicative of but not specific for acetylcholine- and noradrenaline-containing nerves respectively. Although the population of AChE-positive nerves could represent non-specific staining of non-cholinergic nerves, nevertheless a substantial proportion must represent true cholinergic nerves. It is possible that these cholinergic fibres modulate the activity within the adjacent sympathetic nervous system. Such an interaction could potentially be of importance in synchronizing bladder neck closure and the production of prostatic fluid at the time of ejaculation.

In the absence of significant changes in adrenergic or cholinergic innervation in the various study groups it is difficult to explain the increase in noradrenaline content identified on biochemical assay in patients with (unstable or stable) outflow obstruction. This remains an isolated observation which requires further study.

Since it is evident that the male bladder neck receives a significant NANC sensorimotor innervation, it is important to explain the apparent

absence of "sensory" neurotransmitters. A plausible explanation is that nerves immunoreactive to these substances are too sparsely distributed to be identified at microscopy. This suggestion is partially validated by the results of biochemical assay, where significant quantities of CGRP and SP were identified. However, a caveat which must be borne in mind is the discrepancy between the results of biochemical assay and histochemical localisation. For instance, contrasting the results for VIP and substance P, biochemical assay demonstrated concentrations of substance P of a similar magnitude to those obtained for VIP, whereas at microscopy there was significant VIP-immunoreactivity but substance P-immunoreactivity was noticeably absent. This highlights the difficulties inherent in the interpretation of biochemical results since these relate to the tissue content of a neurotransmitter substance irrespective of whether it is lying within nerves or other tissue compartments.

The relevance of the significant reduction in SP content noted within the bladder neck from patients with unstable obstruction, and of the corresponding increase of VIP in the same population, is unclear. Indeed the investigation of bladder neck tissue from a number of different urodynamic subgroups of patients with bladder outflow obstruction can be questioned. Nevertheless, this provided the opportunity not only to investigate the innervation of the bladder neck, but also to review other factors which might be contributing to the alterations in detrusor behaviour seen with prostate obstruction. In particular, it has been suggested that afferent neural

stimulation at the level of the prostate (and therefore presumably bladder neck) could contribute to the genesis of detrusor instability (Chalfin and Bradley 1982).

These histochemical studies demonstrate that the human bladder neck receives a complex neural innervation. On the basis of this evidence it is not possible to draw definite conclusions and complementary functional investigations including in vitro isometric pharmacological studies and slide-mounted autoradiography are essential to allow further interpretation of these results. Nevertheless, whilst the sympathetic nerves acting via noradrenaline release are the principal mediators of contractile activity, it is evident that the functional control of the male bladder neck can potentially be influenced by the interaction of a number of neural populations containing different neurotransmitters.

CHAPTER 12

SUMMARY

This thesis presents the first comprehensive study of either human prostate or bladder tissue in patients with symptomatic bladder outflow obstruction due to benign prostatic enlargement and asymptomatic age matched controls. The techniques used have all been validated independently in previous studies.

The study used a combination of clinical and laboratory investigations.

Techniques

a) Urodynamics

- Measurement of free urinary flow rate.
- Filling and voiding pressure/flow cystometry with simultaneous fluoroscopic imaging and selected video recording, thereby allowing peer review of the results.
- The technique of videocystometry allowed accurate characterisation of the altered functional changes in detrusor behaviour associated with the clinical presentation of obstructive detrusor instability and acute retention of urine.

b) Neurohistochemical

Biopsies of bladder and bladder neck muscle and specimens of prostatic tissue were processed to allow histological and biochemical quantification of a large number of neurotransmitter substances.

Acetylcholinesterase and dopamine β-hydroxylase were used as markers for the classical cholinergic and adrenergic pathways respectively. In

addition, a number of putative neurotransmitter substances were investigated, including vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), somatostatin, substance P, leu-Enkephalin (l-Enk), met-Enkephalin (m-Enk) and 5-hydroxytryptamine (5-HT). The density of nerves containing these various neurotransmitters was assessed both subjectively and objectively using a graticule based point counting technique. The results were expressed as the number of nerves/mm^2 muscle, after correction of the raw data for differences in muscle cell size and number.

Dual staining of nerve fibres for classical autonomic neurotransmitters using acetylcholinesterase and dopamine β-hydroxylase and immunocytochemical staining for a number of putative neurotransmitters associated with the non-adrenergic non-cholinergic (NANC) sensorimotor innervation allowed an overall assessment of the changes in innervation which occur both in the hyperplastic prostate and within the muscle of the obstructed bladder.

c) Pharmacological

- Ligand binding and autoradiographic studies were used to quantify and localise muscarinic cholinoceptors and adrenoceptors (α_1 and α_2) within prostatic tissue.

- The responses of tissues from the bladder and prostate to a variety of neurochemical stimulants were measured by in vitro isometric muscle strip studies.

Methodology

Direct experimental study of the neural control mechanisms of the normal bladder and prostate is not possible in man. Limited information may be obtained by correlating ultrastructural changes in biopsy material with clinical and urodynamic observations. Careful tissue preparation and meticulous immunohistochemical techniques are of utmost importance in obtaining accurate and reproducible visualisation of nerve fibres.

The method used for neural quantification was chosen to minimise sampling errors and the technique has been extensively validated in previous studies of the urinary tract by Gosling and associates. Previous studies of peptide neurotransmitter immunoreactivity have relied on subjective grading of neural density. In this study objective quantification of stained neurones was attempted but the poor reproducibility of the results emphasise the technical difficulties associated with the measurement of such a sparse neural density and support the continued use of subjective quantification.

The pharmacological studies provided a crude assessment of receptor localisation and function, but the use of autoradiography allowed specific receptor localisation in the human prostate. Similar studies on the bladder and bladder neck are in progress.

The selection of patients and in particular the criteria applied to control material is of major importance in a study of this nature. The technique of videocystometry is now established as the gold standard investigation for the assessment of bladder outflow obstruction and provides the most accurate

technique available for the categorisation of patients.

A major weakness of this study lies with the control tissue. There were only two specimens of control tissue for the bladder neck and that obtained for the bladder was limited, a consequence of the strict patient selection criteria. The selection of patients was felt to be important since many previous studies have relied on comparison of normal obstructed tissue to control tissue obtained from patients that had previously undergone radical radiotherapy to the bladder tissue.

The method of electrical stimulation of tissue used in the in vitro pharmacological studies may also be criticised. Previous work has suggested that the pulse duration of the electrical impulse used should be 0.05 msec to avoid direct muscle stimulation. In practice, our observations did not demonstrate a significant component of direct muscle stimulation using a pulse duration of 0.5 msec.

Aetiology of Detrusor Instability in Obstructed Micturition.

The advent and widespread application of urodynamic investigation over the last three decades has permitted the recognition of the importance of functional disturbance of the bladder to the clinical presentation of patients with bladder outlet obstruction. Whilst the response of the bladder is partially dictated by local factors such as its structure and its neural control by local sacral spinal cord reflexes, it is well recognised that detrusor behaviour can be profoundly influenced by the higher centres.

Failure to develop normal bladder control occurs in up to 10% of the population, resulting in idiopathic detrusor instability. Detrusor hyperactivity occurs in at least two-thirds of patients presenting with symptomatic bladder outflow obstruction. Previous hypotheses as to the aetiology of detrusor instability in prostatic obstruction have predominantly concentrated on local changes within the prostate or bladder. I believe that it is wrong to consider these structures out of the context of their central and local reflex control. Nevertheless, since the majority of obstructed patients have no detectable neurological disorder on clinical examination and no history of pre-existing symptoms of detrusor instability prior to the onset of bladder obstruction, it seems unlikely that there is a significant abnormality of central micturition pathways. Detrusor instability secondary to obstruction is usually reversible following relief of the obstruction: marked structural changes occur in the obstructed bladder and a causal link between these two phenomena seems likely.

My aim in carrying out these studies was not only to obtain greater knowledge of the normal structure and function of the human bladder and prostate, but also to try and identify the aetiology of secondary detrusor instability.

The obstructed human bladder has a much reduced innervation as compared to normal tissue. The results clearly demonstrate that this involves all of the innervation and not just the cholinergic nerves as previously suggested. The importance of the non-adrenergic, non-cholinergic

sensorimotor innervation of the bladder remains controversial. This report is the first to compare the neuropeptide distribution within the normal and obstructed detrusor. In addition the localisation of CGRP-immunoreactivity in the human bladder is described for the first time. NPY- and VIP-containing nerves were present in the greatest density, but the physiological role of these substances within the human detrusor remains unclear. Although the reduction in VIP in unstable bladder reported here is consistent with the suggestion that VIP acts as a local inhibitory neuropeptide in the normal bladder, complementary in vitro pharmacological studies are necessary before more definite conclusions can be drawn.

Previous investigators have concentrated principally on the motor innervation of the detrusor muscle and have neglected the importance of the sensory control of detrusor function. In this study, an extensive network of nerves containing sensory neural transmitter substances were found to be localised predominantly to the submucosa and lamina propria of the bladder. A significant reduction in substance P was demonstrated within obstructed detrusor muscle and this has subsequently been reproduced in an animal model (Andersson et al 1988). The physiological role of a putative sensory transmitter such as substance P which also appears to have contractile effects is uncertain. In vitro pharmacological studies of the effect of exogenous putative peptide neurotransmitters are currently in progress.

The importance of structural abnormalities must however be interpreted with caution, since the changes documented in both the bladder and prostate

occured universally in all obstructed patients with no apparent differences between those with stable and unstable detrusor behaviour. Although it is widely assumed that the changes in structure and innervation of the obstructed bladder have a common aetiology. The possibility remains that denervation may be the primary event which results in secondary changes in structure. The suggestion that detrusor instability arises from increased afferent stimulation of the prostatic urethra does not explain the development of such instability in patients with distal obstruction due to a urethral stricture with an apparently normal prostatic urethra, nor does it explain the resolution of instability following local treatment of a stricture.

The pathogenesis of obstruction related detrusor instability is certainly more complex than previously recognised. The results presented here do not provide any evidence of a single difference in detrusor behaviour between stable and unstable groups which would by itself explain the genesis of detrusor instability. It is therefore not suprising that since all previous hypotheses have concentrated on a single abnormality they have failed to explain the disorder. The results presented here support the hypothesis that the functional changes seen in the obstructed bladder represent the expression of non-specific muscular hyperexcitability, which is itself the end result of a number of structural changes. This provides an attractive explanation for the wide variation in detrusor behaviour which can be identified in clinical practice. Further work is required, in particular more extensive mapping of receptor localisation within the normal and obstructed

human detrusor.

The importance of this work rests on its potential therapeutic applications. Non-specific cholinergic antagonists with their accompanying side effects have been the conventional therapy for detrusor instability. The most recent agents have a dual mechanism of action, not only acting as muscarinic antagonists, but also blocking calcium flux and thereby reducing muscle excitability. Increased understanding of the pathophysiology of detrusor instability should allow the development of more specific agents. Our recent clinical study suggests that the long-term use of selective α_1 antagonists may reduce detrusor instability in the obstructed bladder (Chapple et al 1989a). The manipulation of neuropeptide agents may also be of therapeutic benefit; in particular by the use of drugs which selectively influence the sensory limb of local reflex arcs, a subject which forms the basis of ongoing study.

The Neural Control of the Prostate and Bladder Neck in obstructed micturition.

Despite the undoubted importance and complexity of the human prostate gland and adjacent bladder neck, there are surprisingly few studies of their innervation. The human prostate gland fulfils a complex physiological role, and the integrity of the male bladder neck is essential to normal reproductive function. A new more detailed understanding of the underlying neural control mechanisms results from the work presented here and

includes a number of original findings:-

1) The specific autoradiographic localisation of these α_1 adrenoceptors to the stromal compartment provides clear morphological evidence in support of their principal functional importance in prostatic muscle contraction.

2) Muscarinic cholinoceptors are localised specifically to glandular acinar tissue and α_2 adrenoceptors are located on blood vessels and at the basement membrane of glandular acini within the prostate gland.

3) The innervation of the human prostate gland and male bladder neck is complex and contains a number of putative peptide neurotransmitters in addition to the classical adrenergic and cholinergic nerve groups. There are a number of intraprostatic ganglia staining for a variety of different neurotransmitters. It is likely that these allow extensive interaction between different neural populations within the prostate.

4) There is a widespread disturbance of the innervation of the hyperplastic human prostate. Assay of the tissue content of the neurotransmitters noradrenaline, CGRP, Substance P and VIP provides evidence in support of there being a possible disturbance of neural function in the adjacent bladder neck.

The innervation of the human bladder neck and prostate is more complex than previously recognised. The results presented here provide a clear scientific basis for the therapeutic role of specific α_1 adrenoceptor blockade in the medical management of prostate obstruction. Whilst pharmacological studies of the male bladder neck are awaited, the existing

anatomical, histochemical and functional observations suggest that they will mirror the findings obtained for the prostate.

REFERENCES

Abrams, P.H. 1977. The investigation of bladder outflow obstruction in the male. MD Thesis.University of Bristol.

Abrams, P.H. 1985. Detrusor instability in bladder outlet obstruction. Neurourology and Urodynamics. 4:317-328

Abrams, P.H., Blaivas, J.G., Stanton, S.L. and Andersen, J.T. 1988 The standardisation of terminology of lower urinary tract function. Scand. J. Urol. Nephrol. suppl. 114:5-19

Adrian, T.E., Gu, J., Allen, J.M., Tatemoto, K., Polak, J.M. and Bloom, S.R. 1984. Neuropeptide Y in the human male genital tract. Life Sci. 35:2643-2648

Ahlquist, R.P. 1948. A study of the adrenotropic receptors. Am.J. Physiol. 53:586-600

Alm, P., Alumets, J., Hakanson, R. and Sundler, F. 1977. Peptidergic (vasoactive intestinal polypeptide) nerves in the genitourinary tract. Neuroscience. 2:751-754

Alm, P. 1978. Cholinergic innervation of the human urethra and urinary bladder, a histochemical study and review of methodology. Acta.Pharmacol.Toxicol. 43:56-62

Alm, P., Alumets, K., Ek, A. and Sundler, F. 1979. Peptidergic (VIP) nerves in the human urinary tract. Proc.Ninth.Int.Cont.Soc.Meeting Rome:147

Alm, P., Alumets, J., Hakanson, R., Owman, C.L., Sjoberg, N.O., Sundler, F. and Walles, B. 1980. Origin and distribution of vasoactive intestinal polypeptide (VIP) nerves in the genito-urinary tract. Cell Tissue Res. 205:337-347

Ambache, N. 1955. The use and limitations of atropine for pharmacological studies on autonomic effectors. Pharmacol. Rev. 7:467-494

Ambache, N. and Aboo Zar, M.A. 1970. Non-cholinergic transmission by post-ganglionic motor neurones in the mammalian bladder. J. Physiol. (Lond) 210:761-783

Ambache, N., Kilick, S.W. and Woodley, J.P. 1977. Evidence against purinergic motor transmissions in guinea pig urinary bladder. Br.J.Pharmacol. 61:464

Andersen, J.T. 1982. Prostatism III. Detrusor hyperreflexia and residual urine. Clinical and urodynamic aspects and the influence of surgery on the prostate. Scand. J. Urol. Nephrol. 16:25-30

Anderson, G.F. and Navarro, S.P. 1988. The response of autonomic receptors to castration and testostrone in the urinary bladder of the rabbit. J. Urol. 140:885-889 Andersson, K.E. and Sjogren, C. 1982. Aspects on the physiology and pharmacology of the bladder and urethra. Progress in Neurobiology 19:71-89

Andersson, K.E., Mattiasson, A. and Sjogren, C. 1983. Electrically induced relaxation of the noradrenaline contracted isolated urethra from rabbit and man. J. Urol. 129:210-213

Andersson, P.O., Andersson, K.E., Fahrenkrug, J., Mattiasson, A., Sjogren, C. and Uvelius, B. 1988. Contents and effects of substance P and vasoactive intestinal polypeptide in the bladder of rats with and without infravesical outflow obstruction. J. Urol. 140:168-172

Andersson, P.O. and Malmgren, A. 1989. Substance P administration releases acetylcholine from the rat urinary bladder. Unpublished -personal communication
Arnold, E.P. 1973. A urodynamic analysis of detrusor dysfunction. PhD thesis. Univ. of London

Arunlakshana, O. and Schild, H.O. 1959. Some quantitative uses of drug antagonists. Br. J. Pharmacol. 14:48-58

Awad, A.A., Bruce, G., Carrocampi, J.W., Lin, M. and Marks, G.S. 1974. Distribution of α and β adrenoceptors in human urinary bladder. Br. J. Pharmacol. 50:525-529

Ball, A.J., Feneley, R.C.L. and Abrams, P.H. 1981. The natural history of untreated "prostatism". Br. J. Urol. 53:613-616

Barrington, F.J.F. 1914. The nervous mechanism of micturition. Q. J. Exp. Physiol. 8:33-71

Barrington, F.J.F. 1931. The component reflexes of micturition in the cat. Parts 1 & 2. Brain 54:177-188

Barrington, F.J.F. 1941. The component reflexes of micturition in the cat. Part 3. Brain 64:239-243

Bartsch, G., Muller, H.R., Oberholzer, M. and Rohr, H.P. 1979. Light microscopic stereological analysis of the normal human prostate and of benign prostatic hyperplasia. J. Urol. 122:487-491

Bates, C.P., Whiteside, C.G. and Turner-Warwick, R.T. 1970. Synchronous cine/pressure/flow/cysto-urethrography with special reference to stress and urge incontinence. Br. J. Urol. 42:714-723

Bates, C.P. 1971a. Continence and incontinence. A clinical study of the dynamics of voiding and of the sphincter mechanism. Ann. R. Coll. Surg. Engl. 49:18-35

Bates, C.P. and Corney, C.E. 1971b. Synchronous cine/pressure/flow cystourethrography: a method of routine urodynamic assessment. Br. J. Radiol. 44:44-50

Bates, C.P. 1973. Continence and incontinence. D.M.Thesis., University of Oxford.

Baumgarten, H.G., Falck, B., Holstein, A.F., Owman, C.H. and Owman, T. 1968. Adrenergic innervation of the human testis epididymis ductus deferens and prostate. A fluorescence microscopic and fluorimetric study. Z. Zellforsch 90:81-95

Beaumont, A. 1983. Putative peptide neurotransmitters. The opioid peptides. Int. Rev. Exp. Pathol. 25:279-305

Belai, A., Lincoln, J., Milner, P., Crowe, R., Loesch, A. and Burnstock, G. 1985. Enteric nerves in diabetic rats increase in vasoactive intestinal polypeptide but not substance P. Gastroenterology 89:967-976

Benoit, G., Quillard, J. and Jardin, A. 1988, Anatomical study of the inframontanal urethra in man. J. Urol. 139:866-868

Benson, G.S., Raezer, D.M., Wein, A.J. and Carriere, J.N. 1975. Effect of muscle length on adrenergic stimulation of canine detrusor. Urol. 5:769-772

Benson, G.S., McConnell, J.A. and Wood, J.G. 1979, Adrenergic innervation of the human bladder body. J. Urol. 122:189-191

Bo, X. and Burnstock, G. 1990, [^{3}H]-α, β-Methylene ATP, a radioligand labelling P^2-purinoceptors. Journal of the Autonomic Nervous System. 28:85-88

Booth, A.M., Ostrowski, N., McLinden, S., Lowe, I. and De Groat, W.C. 1981. An analysis of the inhibitory effects of leucine enkephalin on transmission in vesical parasympathetic ganglia of the cat. Society for Neuroscience Symposia Abstract 8:214

Brent, L. and Stephens, F.D. 1975. The response of smooth muscle cells in the rabbit urinary bladder to outflow obstruction. Invest. Urol. 12:494-502

Bruschini, H., Schmidt, R.A. and Tanagho, E.A. 1978. Neurologic control of the prostatic secretion in the dog. Invest. Urol. 15:288-290

Bulbring, E. 1953. Measurements of oxygen consumption in smooth muscle. J. Physiol. 122:111-134

Burn, J.H. and Rand, M.J. 1965. Acetylcholine in adrenergic transmission. Ann. Rev. Pharmacol. 5:163-182

Burnstock, G. 1969. Evolution of the autonomic innervation of visceral and cardiovascular systems in vertebrates. Pharmacol. Rev. 21:247-324

Burnstock, G. 1972. Purinergic nerves. Pharmacol.Rev. 24:509-581

Burnstock, G., Cocks, T., Crowe, R. and Kasakov, L. 1978. Purinergic innervation of the guinea pig urinary bladder. Br. J. Pharmacol. 63:125-138

Burnstock, G. 1986a. The changing face of autonomic neurotransmission. Acta physiol. Scand. 126:67-91

Burnstock, G. 1986b. Autonomic neuromuscular junctions: Current developments and future directions. J. Anat. 146:1-30

Burt, A.M. and Silver, A. 1973. Histochemistry of choline acetyl transferase: a critical analysis. Brain. Res. 62:509-516

Caine, M. 1986a. Clinical experience with α-adrenoceptor antagonists in benign prostatic hypertrophy. Fed. Proc. 45:2604-2608

Caine, M. 1986b. The present role of α adrenergic blockers in the treatment of benign prostatic hypertrophy. J. Urol. 136:1-4

Caine, M., Raz, S. and Zeigler, M. 1975. Adrenergic and cholinergic receptors in the human prostate, prostatic capsule and bladder neck. Br. J. Urol. 47:193-202

Cannon, W.B., Rosenblueth, A., 1949. The supersensitivity of denervated structures: a law of denervation. NewYork, MacMillan, 245p.

Carpenter, F.G. and Rand, S.A. 1965. Relation of acetylcholine release to responses of the rat urinary bladder. J. Physiol. (Lond) 180:371-382

Carpenter, F.G. and Rubin, R.M. 1967. The motor innervation of the rat urinary bladder. J. Physiol. 192:609-617

Casas, A.P. 1958. Die innervation der menschlichen vorsteherdruse. Zschr. Mikrosk. Anat. Forsch. 64:608-633

Chalfin, S.A. and Bradley, W.E. 1982. The aetiology of detrusor hyperreflexia in patients with infravesical obstruction. J. Urol. 127:938-942

Chapple, C.R., Christmas, T.J. and Milroy, E.J.G. 1989a. A study of the long-term use of the selective α-1 antagonist prazosin in the management of benign prostatic hyperplasia. Urol. Int. (in press).

Chapple, C.R., Helm, C.W., Blease, S., Milroy, E.J.G., Rickards, D. and Osborne, J.L. 1989b. Asymptomatic bladder neck incompetence in nulliparous females. Br. J. Urol. 64:357-359

Chubb, I.W., Hodgson, A.J. and White, G.H. 1980. Acetylcholinesterase hydrolyses substance P. Neuroscience 5:2065-2072

Chun, A.L., Wallace, L.J., Gerald, M.C., Levin, R.M. and Wein, A.J. 1988. Effect of age on in-vitro urinary bladder function in the rat. J. Urol. 139:625-627

Claridge, M. 1966. Analyses of obstructed micturition. Ann. R. Coll. Surg. 39:30-53

Coolsaet, B.L.R.A., Van Venroois, G.E.P.M. and Blok, C. 1984. Prostatism: Rationalisation of urodynamic testing. World J. Urol. 2:216-221

Coons, A.H., Leduc, E.H. and Connolly, J.H. 1955. Studies on antibody production 1. A method for the histochemical demonstration of specific antibody and its application to a study of the hyperimmune rabbit. J. Exp. Biol. 102:49-59

Corrodi, H. and Jonsson, G. 1967. The formaldehyde fluorescence method for the histochemical demonstration of biogenic amines. A review on the methodology. J. Histochem. Cytochem. 15:65-78

Cowan, W.D. and Daniel, E.E. 1983. Human female bladder and its non-cholinergic contractile function. Can. J. Physiol. Pharmacol. 61:1236-1246

Craggs, M.D. and Stephenson, J.D. 1985. Bladder electromyograms and function in monkeys after atropine. Br. J. Urol. 57:341-345

Creevy, C.D. 1934. Distension of the urinary bladder. 1. Haematuria and sudden emptying; an experimental and clinical study. Arch. Surg. 28:948-973

Crowe, R., Burnstock, G. and Light, J.K. 1988. Intramural ganglia in the human urethra. J. Urol. 139:140-183

Crowe, R., Chapple, C.R., Milroy, E.J.G., Turner-Warwick, R.T. and Burnstock, G. 1989. The human prostate gland: a histochemical and immunohistochemical study of neuropeptides, serotonin, dopamine-β-hydroxylase and acetylcholinesterase in autonomic nerves and ganglia. (J.Urol. submitted for publication)

Crowe, R., Moss, H., Chapple, C.R., Light, J.K., Burnstock, G. 1990. Patients with lower motor spinal cord lesions: A decrease of vasoactive intestinal polypeptide, calcitonin gene-related peptide and substance P, but not neuropeptide Y and somatostatin-immunoreactive nerves in the detrusor muscle of the bladder. (J.Urol. submitted for publication)

Cullumbine, H., McKee, W.H.E. and Creasey, N.H. 1955. The effects of atropine sulphate upon healthy male subjects. Q. J. Exp. Physiol. 30:309-319

Dale, H.H. 1933. Nomenclature of fibres in the autonomic system and their effects. J. Physiol. (Lond) 80:10p-11p

Dale, H.H. and Gaddum, J.H. 1930. Reactions of denervated voluntary muscle and their bearing on the mode of action of parasympathetic and related nerves. J. Physiol. (Lond) 70:109-144

Daniel, E.E., Cowan, W. and Daniel, V.P. 1983. Structural bases for neural and myogenic control of human detrusor muscle. Can. J. Physiol. Pharmacol. 61:1247-1273

Dean, D.M. and Downie, J.W. 1978. Contribution of adrenergic and "purinergic" neurotransmission to contraction in rabbit detrusor. J. Pharmacol. Exp. Ther. 207:431-445

De Groat, W.C. and Booth, A.M. 1980. Inhibition and facilitation in parasympathetic ganglia of the urinary bladder. Fed. Proc. 39:2990-2996

De Groat, W.C. and Kawatani, M. 1985. Neural control of the urinary bladder: Possible relationship between peptidergic inhibitory mechanisms and detrusor instability. Neurourology and Urodynamics 4:285-300

De May. J.G. and Vanhoutte, P.M. 1980. Differences in pharmacological properties of postjunctional α-2 adrenergic receptors among arteries and veins.. Arch. Int. Pharmacodyn. Ther. 224:328-329

Denny-Brown, D. and Robertson, E.G. 1933a. On the physiology of micturition. Brain 56:149-190

Denny-Brown, D. and Robertson, E.G. 1933b. The state of the bladder and its sphincters in complete transverse lesions of the spinal cord and cauda equina. Brain 56:397-462

Desnos, E. 1914. L'histoire de l'urologie. , Thomas, Illinois, translation in "The history of urology" ed. L.T.J. Murphy (1972).

Dion, S., Corcos, J., Carmel, M., Drapeau, G. and Regoli, D. 1988. Substance P and neurokinins as stimulants of the human isolated urinary bladder. Neuropeptides 11:83-87

Di Sant'Agnese, P.A.and De Mesy Jensen, K.L. 1984. Endocrine-Paracrine cells of the prostate and prostatic urethra. Human Pathology 15:1034-1041

Di Sant'Agnese, P.A., De Mesy Jensen, K.L., Churukian, C.J. and Agarwal, M.M. 1985. Human prostatic endocrine-paracrine (APUD) cells. Arch. Path. 109:607-612

Dixon, J.S., Gilpin, S.A., Gilpin, C.G. and Gosling, J.A. 1983. Intramural ganglia of the human urinary bladder. Br. J. Urol. 55:195-198

Dixon, J.S. and Gosling, J.A. 1983. Histology and fine structure of the muscularis mucosae of the human urinary bladder. J. Anat. 136:265-271

Dixon, J.S. and Gosling, J.A. 1987. Chapter 1. Structure and innervation in the human. In: Physiology in the lower urinary tract. Torrens, M. and Morrison, J.F.B., Eds., London, Springer Verlag, 3-22

Donker, P.J., Ivanovici, F. and Noach, E.L. 1972. Analyses of the urethral pressure profile by means of electromyography and the administration of drugs. Br. J. Urol. 44:180-193

Doyle, P.T. and Hill, D.W. 1976. Detrusor electomyography. In: Scientific Foundations of Urology. Williams,D.J. and Chisholm,G.D., Eds., Heinemann.

Drew, G.M. 1978. Pharmacological characterisation of the presynaptic α-adrenoceptors regulating cholinergic activity in the guinea-pig ileum. Br. J. Pharmacol. 64:293-300

Dubois, P. 1876. Ueber den Druck in der Harnblase. Dt. Arch. Klin. Med. 17:148-163

Dumsday, B. 1971. Atropine resistance of the urinary bladder innervation. J. Pharm. Pharmacol. 23:222-225

Duncan, G.W. and Goodwin, W.E., 1949. Experimental urinary tract obstruction produced by cellophane sclerosis. Surgery 25:113-116

Dunn, M. 1974. A study of the bladder blood flow during distension in rabbits. Br. J. Urol. 46:67-72

Dunzendorfer, U., Jonas, D. and Weber, W. 1976. The autonomic innervation of the human prostate. Histochemistry of acetylcholinesterase in the normal and pathologic states. Urol. Res. 4:29-31

Eaton, A.C. and Bates, C.P. 1982. An in vitro physiological study of normal and unstable human detrusor muscle. Br. J. Urol. 54:653-657

Edge, N.D. 1955. A contribution to the innervation of the urinary bladder of the cat. J. Physiol. (Lond) 127:54-68

Edvarsen, P. 1968a. Nervous control of urinary bladder in cats. 1. The collecting phase. Acta Physiol. Scand. 72:157-171

Edvardsen. P. 1968b. Nervous control of urinary bladder in cats 111. Effects of autonomic blocking agents in the intact animal. Acta Physiol. Scand. 99:345-352

Edvardsen, P. and Setekleiv, J. 1968. Distribution of adrenergic receptors in the urinary bladder of cats, rabbits and guinea pigs. Acta Pharmacol. et Toxicol. 26:437-445

Ek, A., Alm, P., Andersson, K.E. and Persson, C.G.A. 1977. Adrenergic and cholinergic nerves of the human urethra and urinary bladder. A histochemical study. Acta Physiol. Scand. 99:345-352

Ek, A., Andersson, K.E. and Ulmsten, U. 1978. The effects of norephedrine and bethanecol on the human urethral closure pressure profile. Scand. J. Urol. Nephrol. 12:97-104

Ekstrom, J., Malmberg, L. and Wallin, A. 1986. Transient supersensitivity in the hypertrophied rat urinary bladder. Acta Physiol. Scand. 126:365-370

Elbadawi, A. and Schenk, E.A. 1966. Dual innervation of the mammalian urinary bladder. A histochemical study of the distribution of cholinergic and adrenergic nerves. Am. J. Anat. 119:405-416

Elbadawi, A. 1982. Neuromorphologic basis of vesico-urethral function; 1 Histochemistry, ultrastructure and function of intrinsic nerves of the bladder and urethra. Neurourology and Urodynamics 1:3-50

Elliott, T.R. 1907. The innervation of the bladder and urethra. J. Physiol. (Lond) 25:367-445

Elmer, M. 1974. Action of drugs on the innervated and denervated urinary bladder of the cat. Acta Physol. Scand. 91:289-297

Erspamer, V., Ronzoni, G. and Falconieri-Erspamer, G. 1981. Effects of active peptides on the isolated muscle of the human urinary bladder. Invest. Urol. 18:302-304

Feher, E., Hosszu, E. and Vajda, J. 1982. Distribution of primary sensory nerve terminals in the cat urinary bladder wall. Acta. Morph. Acad. Sci. Hung. 30:223-232

Fitzpatrick, J.M., Gardiner, R.A. and Worth, P.H.L. 1979. The evaluation of 68 patients with post-prostatectomy incontinence. Br. J. Urol. 51:552-555

Fleming, W.W., McPhillips, J.J. and Westfall, D.P. 1973. Postjunctional supersensitivity and subsensitivity of excitable tissues to drugs. Rev. Physiol. 68:55-119

Fleming, W.W. and Westfall, D.P. 1975. Altered resting membrane potential in the supersensitive vas deferens of the guinea pig. J. Pharmac. Exp. Ther. 192:381-389

Fletcher, T.F. and Bradley, W.E. 1969. Comparative morphological features of urinary bladder innervation. Am. J. Vet. Res. 30:1655-1662

Fletcher, T.F. and Bradley, W.E. 1970. Afferent nerve endings in the urinary bladder of the cat. Am. J. Anat. 128:147-158

Fonnum, F. 1975. A rapid radiochemical method for the determination of choline acetyltransferase. J. Neurochem. 24:407-409

Franks, L.M. 1954. Benign nodular hyperplasia of the prostate: a review. Ann. R. Coll. Surg. Engl. 14:92-106

Frimodt-Moller, C. 1972. A new method for quantative evaluation of bladder sensibility. Scand. J. Urol. Nephrol. 6(Suppl.15):135-142

Frimodt-Moller, P.C., Jensen, K.M.E., Iversen, P., Madsen, P.O. and Bruskewitz, R.C. 1984. Analysis of presenting symptoms in prostatism. J. Urol. 132:272-276

Furuya, S., Kumamoto, Y., Yokoyama, E., Tsukamoto, T., Izumi, T. and Abiko, Y. 1982. Alpha-adrenergic activity and urethral pressure profilometry in prostatic zone in benign prostatic hypertrophy. J. Urol. 128:836-839

George, M.J.R. and Dixon, J.S. 1986. In sensory disorders of the bladder and urethra. George, N.J.R., Gosling, J.A.,Eds. Springer-Verlag. London. Berlin.

Ghoneim, G.M., Regnier, C.H., Biancani, P., Johnson, L. and Susset, J.G. 1986. Effect of vesical outlet obstruction on detrusor contractility and passive properties in rabbits. J. Urol. 135:1284-1289

Gibbins, I.L., Furness, J.B., Costa, A., McIntyre, I., Hillyard, C.J. and Girgis, S. 1985. Co-localisation of calcitonin gene related petide immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurones of guinea-pigs. Neurosci. Lett. 57:125-130

Gibbins, I.L., Furness, J.B. and Costa, M. 1987. Pathway-specific patterns of the co-existence of substance P, calcitonin gene-related peptide, cholecystokinin and dynorphin in neurones of the dorsal root ganglia of the guinea pig. Cell Tissue Res. 248:417-437

Gilpin, C.J., Dixon, J.S., Gilpin, S.A. and Gosling, J.A. 1983. The fine structure of autonomic neurones in the wall of the human urinary bladder. J. Anat. 137:705-713

Gilpin, S.A., Gosling, J.A. and Barnard, R.J. 1985. Morphological and morphometric studies of the human obstructed trabeculated urinary bladder. J. Urol. 57:525-529

Gilpin, S.A., Gilpin, C.J., Dixon, J.S., Gosling, J.A. and Kirby, R.S. 1986. The effect of age on the autonomic innervation of the urinary bladder. B. J. Urol. 58:378-381

Glynn, R.J., Campion, E.W., Bouchard, G.R. and Silbert, J.E. 1985. The development of benign prostatic hyperplasia among volunteers in the normative aging study. Am. J. Epidemiol. 121:78-90

Gomori, G. 1952. Microscopy histochemistry - principles and practice. Chicago. University of Chicago Press. :208-214

Goodman, E.C. and Iverson, L.L. 1986. Calcitonin gene related peptide: novel neuropeptide. Life Sci. 38:2169-2178

Gosling, J.A. and Dixon, J.S. 1974. Sensory nerves in the mammalian urinary tract. An evaluation using light and electron microscopy. J. Anat. 117:133-144

Gosling, J.A. and Dixon, J.S. 1975. Structure and innervation of smooth muscle in the wall of the bladder neck and proximal urethra. J. Urol.47:549-558

Gosling, J.A., Dixon, J.S. and Dunn, M. 1977. The structure of the rabbit urinary bladder after experimental distension. Invest. Urol. 14:386-389

Gosling, J.A., Dixon, J.S. and Lendon, R.G. 1977. The autonomic innervation of the human male and female bladder neck and proximal urethra. J. Urol. 118:302-305

Gosling, J.A. and Thompson, S.A. 1977. A neurohistochemical and histological of peripheral autonomic neurones of the human bladder neck and prostate. Urol. Int. 32:269-276

Gosling, J.A. 1979. The structure of the bladder and urethra in relation to function. Urol. Clin. North Am. 6:31-38

Gosling, J.A. and Dixon, J.S. 1980. Structure of trabeculated detrusor smooth muscle in cases of prostatic hypertrophy. Urol. Int. 35:351-355

Gosling, J.A., Dixon, J.S. and Humpherson, J.R. 1983. Functional anatomy of the urinary tract. London, Churchill Livingstone.

Gosling, J.A. 1983. Autonomic innervation of the prostate. In Benign Prostatic Hypertrophy. Hinman, F.,Jr Ed. Springer Verlag, Berlin. p349-360

Gosling, J.A. 1986. The distribution of noradrenergic nerves in the human lower urinary tract. Clin. Sci. 70:3s-6s

Gosling, J.A., Gilpin, S.A., Dixon, J.S. and Gilpin, C.J. 1986. Decrease in the autonomic innvervation of human detrusor muscle in outflow obstruction. J. Urol. 136:501-503

Griffiths, J. 1891. Observations on the urinary bladder and urethra. J. Anat. Physiol. 25:535-549

Gu, J., Restorick, J.M., Blank, M.A., Huang, W.M., Polak, J.M., Bloom, S.R. and Mundy, A.R. 1983a. Vasoactive intestinal polypeptide in the normal and unstable bladder. Br. J. Urol. 55:645-647

Gu, J., Polak, J.M. and Polak, P.L. 1983b. Peptidergic innvervation of the human genital tract. J. Urol. 130:386-391

Gu, J., Blank, M.A., Huang, W.M., Islam, K.N., McGregor, G.P., Christofides, N., Allen, J.M., Bloom, J.R. and Polak, J.M. 1984. Peptide containing nerves in human urinary bladder. Urology. 24:353-357

Guyon, F. and Albarron, J. 1890. Anatomie et physiologie pathologiques de la retention d'urine. Arch de med. exper. et d'anat. path. 2:181-221

Hakanson, R., Larsson, L.I., Sjoberg, N.O. and Sundler, F. 1974. Amine-producing endocrine-like cells in the epithelium of urethra and prostate of the guinea-pig a chemical, fluorescence, histochemical and electron microscopic study. Histochemistry 38:259-270

Hald, T. 1969. Neurogenic dysfunction of the urinary bladder. Thesis. University of Copenhagen

Hamberger, B. and Norberg, K.A. 1965. Adrenergic synaptic terminals and nerve cells in bladder ganglia of the cat. Int. J. Neuropharmacol. 4:41-45

Harrison, S.C.W., Hunnam, G.R., Farman, P., Ferguson, D.R. and Doyle, P.T. 1987. Bladder instability and denervation in patients with bladder outflow obstruction. Br. J. Urol. 60:519-522

Hedlund, H., Andersson, K.E. and Ek, A. 1983. Effects of prazosin in patients with benign prostatic obstruction. J. Urol. 130:275-278

Hedlund, H., Andersson, K.E. and Larsson, B. 1985. Alpha-adrenoceptors and muscarinic receptors in the isolated human prostate. J. Urol. 134:1291-1298

Henderson, V.E. and Roepke, M.H. 1934. The role of acetylcholine in bladder contractile mechanisms and in parasympathetic ganglia. J. Pharmacol. Exp. Ther. 51:97-111

Henderson, V.E. and Roepke, M.H. 1935. The urinary bladder mechanisms. J. Pharmacol. Exp. Ther. 54:408-414

Hervonen, A. 1971. Development of catecholamine - storing cells in human foetal paraganglia and adrenal medulla. Acta Physiol. Scand. Suppl. 368:1-94

Hervonen, A., Vaalasti, A., Partanen, M., Kanerva, L. and Hervonen, H. 1978. Effects of ageing on the histochemically demonstrable catecholamines and acetylcholinesterase neurones of human sympathetic ganglia. J. Neurocytol. 7:11-23

Hieble, J.P., Caine, M. and Zalaznik, E. 1985. In vitro characterization of the α adrenoceptors in human prostate. Eur. J. Pharmacol. 107:111-117

Hieble, J.P. and Caine, M. 1986. Aetiology of benign prostatic hyperplasia and approaches to its pharmacological management. Fed. Proc. 45:2601-2603

Higgins, J.R.A., Gosling, J. and Blacklock, N.J. 1989. Studies on the structure and innervation of the prostate and changes in association with benign prostatic hyperplasia. Presented at the annual meeting of the British Association of Urological Surgeons

Higson, R.H., Smith, J.C. and Hills, W. 1979. Intravesical lignocaine and detrusor instability. Br. J. Urol. 51:500-503

Hills, J., Meldrum, L.A., Klarskov, P. and Burnstock, G. 1984. A novel non-adrenergic, non-cholinergic nerve mediated relaxation of the pig bladder neck; an examination of possible neurotransmitter candidates. Eur. J. Pharmacol. 99:287-293

Hindmarsh, J.R., Idowu, A.O., Yeates, W.W. and Aboo Zar, M.A. 1977. Pharmacology of electrically evoked contraction of the bladder. Br. J. Pharmacol. 61:115P

Hirst, G.D.S.and Neild, T.O. 1980. Evidence for two populations of excitatory receptors for noradrenaline on arteriolar smooth muscle. Nature. 283:767-768

Hisamitsu, T., Roques, B.P. and De Groat, W.C. 1982. The role of enkephalins in the sacral parasympathetic reflex pathways to the urinary bladder of the cat. Society for Neuroscience Symposia Abstract 8:227

Hodgkinson, C.P., Ayers, M.A. and Drukker, B.H. 1963. Dyssynergic detrusor dysfunction in the apparently normal female. Am. J. Obstet. Gynec. 87:717-730

Hokfelt, T., Elfvin, L.G., Elde, R., Schultzberg, M., Goldstein, M. and Luft, R. 1977. Occurrence of somatostatin-like immunoreactivity in some peripheral sympathetic noradrenergic neurons. Proc. Natl. Acad. Sci. 74:3587-3591

Hokfelt, T., Johansson, O., Ljungdahl, A., Lundberg, J.M. and Schultzberg, M. 1980. Peptidergic neurones. Nature. 284:515-521

Holt, S.E., Cooper, M. and Wyllie, J.H. 1985. Evidence for purinergic transmission in mouse bladder and for modulation of responses to electrical stimulation by 5-hydroxytryptamine. Eur. J. Pharmacol. 116:105-111

Hoyle, C.H.V, Chapple, C.R., Burnstock, G. 1989. Isolated human bladder: evidence for an adenine dinucleotide acting on P_{2x}-purinoceptors and for purinergic transmission. Eur. J. Pharmacol. 174:115-118

Hunter, D.T. 1954. A new concept of urinary bladder musculature. J. Urol. 71:695-704

Hunter, J. 1786. Treatise on venereal disease. In: The works of John Hunter FRS. Vol 11., Palmer, J.F., Ed., Paternoster Row London, Longman Rees Orme Brown Green and Longman, 1835, p284

Husted, S., Sjogren, C. and Andersson, K.E. 1983. Direct effects of adenosine and adenine nucleotides on isolated human urinary bladder and their influence on electrically induced contractions. J. Urol. 130:392-398

Hutch, J.A. 1963. Ureteric advancement operation: anatomy technique and early results. J. Urol. 89:180-184

Hutch, J.A. and Rambo, O.N.Jr. 1970. A study of the anatomy of the prostate, prostatic urethra and the urinary sphincter system. J. Urol. 104:443-452

Iggo, A. 1955. Tension receptors in the stomach and the urinary bladder. J. Physiol. 128:593-607

Ingersoll, E.H., Jones, L.L. and Hegre, E.S. 1954. Urinary bladder response to unilateral stimulation of hypogastric nerves. J. Urol. 72:178-190

Ingersoll, E.H. and Jones, L.L. 1958. Effect upon the urinary bladder of unilateral stimulation of hypogastric nerves in the dog. Anat. Rec. 130:605-615

Ingersoll, E.H. and Jones, L.L. 1962. Effect of stimulation of autonomic outflow to urinary bladder of rhesus monkey. Pro. Soc. Exp. Biol. Med. 110:858-861

Jancso, G., Kiraly, E. and Jancso-Gabor, A. 1977. Pharmacologically induced selective degeneration of chemo-sensitive primary sensory neurons. Nature. 270:741-742

Jancso, G., Kiraly, E., Joo, F., Such, G. and Nagy, A. 1985. Selective degeneration of a subpopulation of primary sensory neurones in the adult rat. Neurosci. Lett. 59:209-214

Jauernig, R.A., Moulds, R.F.W. and Shaw, J. 1978. The action of prazosin in human vascular preparations. Arch. Int. Pharmacodyn. Ther. 231:81-89

Jensen, D. 1981. Pharmacological studies of the uninhibited neurogenic bladder. Acta Neurol. Scand. 64:175-195

Johns, A. 1979. The effect of VIP on the urinary bladder and taenia coli of the guinea-pig. Can. J. Physiol. Pharmacol. 57:106-108

Jorgensen, T.M., Djorhuus, J.C., Jorgensen, H.S. and Sorensen, S.S. 1983. Experimental bladder hyperreflexia in pigs. Urol. Res. 11:239-240

Jungblut, T., Melchior, H., Aumueller, G. and Malek, B. 1989. Age-dependency and regional distribution of enkephalinergic nerves in human prostate. J. Urol. 141:307A

Kalbfleisch, R.E. and Daniel, E.E. 1987. The role of substance P in the human urinary bladder. Arch. Int. Pharmacodyn. 285:238-248

Karnovsky, M.J. and Roots, L. 1964. A direct colouring method for cholinesterase. J. Histochem. Cytochem. 12:219-221

Kato, K., Wein, A.J., Kitada, S., Haugaard, N. and Levin, R.M. 1988. The functional effect of mild outlet obstruction in the rabbit urinary bladder. J. Urol. 140:880-884

Kawatani, M., Rutigliano, M. and de Groat, W.C. 1985. Selective facilitatory effect of vasoactive intestinal polypeptide (VIP) on muscarinic firing in vesical ganglia of the cat. Brain Res. 336:223-234

Keller, R., Oke, A., Mefford, I. and Adams, R.N. 1976. Liquid chromatographic analysis of catecholamines: routine assay for regional brain mapping. Life Sci. 19:995-1004

Kennedy, C., Saville, V.L. and Burnstock, G. 1986. The contributions of noradrenaline and ATP to the responses of the rabbit central ear artery to sympathetic nerve stimulation depend on the parameters of stimulation. Eur. J. Pharmacol. 122:291-300

Kiesswetter, H. 1977. Mucosal sensory threshold of the urinary bladder and urethra measured electrically. Urol. Int. 32:437-448

Kinder, R.B. and Mundy, A.R. 1985a. Inhibition of spontaneous contractile activity in isolated human detrusor muscle strips by vasoactive intestinal polypeptide. Br. J. Urol. 57:20-23

Kinder, R.B. and Mundy, A.R. 1985b. Atropine blockade of nerve-mediated stimulation of the human detrusor. Br. J. Urol. 57:418-421

Kinder, R.B., Restorick, J.M. and Mundy, A.R. 1985c. Vasoactive intestinal polypeptide in the hyperreflexic neuropathic bladder. Br. J. Urol. 57:289-291

Kinder, R.B. and Mundy, A.R. 1987. Pathophysiology of idiopathic detrusor instability and detrusor hyperreflexia an in vitro study of human detrusor muscle. Br. J. Urol. 60:509-515

Kiowski, W., Hulthen, L., Ritz, R. and Buhler, F.R. 1983. Alpha-2 adrenoceptor-mediated vasoconstriction of arteries. Clin. Pharmacol. Ther. 34:565-569

Kirby, R.S. 1987. MD Thesis. University of Cambridge.

Kirby, R.S., Coppinger, S.W.C., Corcoran, M.O., Chapple, C.R., Flannigan, M. and Milroy, E.J.G. 1987. Prazosin in the treatment of prostatic obstruction - a placebo controlled trial. Br. J. Urol. 60:136-142

Kitada, S. and Kumazawa, J. 1987. Pharmacological characteristics of smooth muscle in benign prostatic hyperplasia and normal prostatic tissue. J. Urol. 138:158-160

Klarskov, P., Gerstenberg, T., Ramirez, D. and Hald, T. 1983. Non-cholinergic, non-adrenergic nerve mediated relaxation in trigone, bladder neck and urethral smooth muscle invitro. J. Urol. 129:848-850

Klarskov, P., Gerstenberg, T. and Hald, T. 1984a. Vasoactive intestinal polypeptide influence on lower urinary tract smooth muscle from human and pig. J. Urol. 131:1000-1004

Klarskov, P., Fahrenkrug, J., Holm-Bentzen, M., Norgaard, T., Ottersen, B., Walter, S. and Hald, T. 1984b. Vasoactive intestinal polypeptide (VIP) concentration in bladder neck, smooth muscle and VIP influence on urodynamic parameters. Proc. 14th Meeting of the Int. Cont. Soc. :228-230

Klarskov, P. and Horby-Petersen, J. 1986. The influence of serotonin on lower urinary tract smooth muscle in-vitro. Br. J. Urol. 58:507-513

Klarskov, P. 1987a. Enkephalin inhibits presynaptically the contractility of urinary tract smooth muscle. Br. J. Urol. 59:31-35

Klarskov, P. 1987b. Non-cholinergic non-adrenergic nerve mediated relaxation of pig and human detrusor muscle in vitro. Br. J. Urol. 59:414-419

Klarskov, P., Holm-Bentzen, M., Norgaard, T., Ottesen, B., Walter, S. and Hald, T. 1987c. Vasoactive intestinal polypeptide concentration in human bladder neck smooth muscle and its influence on urodynamic parameters. Br. J. Urol. 60:113-118

Klarskov, P. 1987d. Non-cholinergic non-adrenergic inhibitory nerve responses of bladder outlet smooth muscle - in vitro. Br. J. Urol. 60:337-342

Klevmark, B. 1974. Motility of the urinary bladder in cats during filling at physiological rates 1 Intravesical pressure patterns studied by a new method of cystometry. Acta Physiol. Scand. 90:565-577

Klevmark, B. 1977. Motility of the urinary bladder in cats during filling at physiological rates 11. Effects of extrinsic bladder denervation on intravesical pressure patterns. Acta Physiol. Scand. 101:176-184

Kleyntjens, F. and Langworthy, O. 1937. Sensory nerve endings on the smooth muscle of the urinary bladder. Journal of Comparative Neurology. 67:367-380

Kluck, P. 1980. The autonomic innervation of the human urinary bladder, bladder neck and urethra; A histochemical study. Anat. Rec. 198:439-447

Koelle, B.G. and Friedenwald, J.S. 1949. A histochemical method for localising cholinesterase activity. Proc. Soc. Exp. Biol(NY). 70:617-622

Koelle, G.B. 1951. The elimination of enzymatic diffusion artefacts in the histochemical localisation of cholinesterases and a survey of cellular distributions. J. Pharmacol. Exp. Ther. 103:153-171

Kohlraush, 1854. Zur anatomie und physiologie der Beckenorgane. in: The anatomy and function of the bladder neck. Tanagho, E.A., Smith, D.R., Br. J. Urol.,1966. 38:54-71

Kolta, M.G., Wallace, L.J. and Gerald, M.C. 1984, Age related changes in sensitivity of rabbit urinary bladder to autonomic agents. Mechanisms of ageing and development 27:183-188

Kondo, M., Taniyama, K. and Tanaka, C. 1985. Histamine H1-receptors in the guinea-pig urinary bladder. Eur. J. Pharmacol. 114:89-92

Kunisawa, Y., Kawabe, K. and Niijima, T. 1985. A pharmacological study of α adrenoceptor sub-types in smooth muscle of human urinary bladder base and prostatic urethra. J.Urol. 134:396-398

Kuru, M. 1965. Nervous control of micturition. Physiol. Rev. 45:425-494

LaMotte, C.C. and De Lanerolle, N.C. 1981. Human spinal neurons innervation by both substance P and enkephalin. Neuroscience 6:713-723

Langer, S.Z. 1974. Presynaptic regulation of catecholamine release. Biochem. Pharmacol. 23:1793-1800

Langley, J.N. and Anderson, H.K. 1894. The constituents of the hypogastric nerves. J. Physiol. 17:177-191

Langley, J.N. and Anderson, H.K. 1895a. The innervation of the pelvic and adjoining viscera. Part 2. The bladder. J. Physiol. (Lond) 19:85-139

Langley, J.N. and Anderson, H.K. 1895b. The innervation of the pelvic and adjoining viscera. Part 1. J. Physiol. (Lond) 19:71-84

Langley, J.N. and Anderson, H.K. 1895c. The innervation of the pelvic and adjoining viscera. Part 4. The internal generative organs. Part 5. Position of the nerve cells on the course of the efferent nerve fibres. J. Physiol. 18:122-139

Langley, J.N. and Anderson, H.K. 1896. The innervation of the pelvic and adjoining viscera .Part 6. Anatomical observations. J. Physiol. 20:372-406

Larsen, J.J. 1979. α and β adrenoceptors in the detrusor muscle and bladder base of the pig and β adenoceptors in the detrusor muscle of man. Br. J. Pharmacol. 65:215-222

Larsen, J.J., Ottenson, B., Fahrenkrug, J. and Fahrenkrug, L. 1981. Vasoactive polypeptide (VIP) in the male genitourinary tract. Concentration and motor effect. Invest. Urol. 19:211-213

Larsson, L.I., Fahrenkrug, J., Schaffalitzkyde de Muckadell, O.B. 1977. Occurrence of nerves containing VIP-immunoreactivity in the male genital tract. Life Sciences 21:503-508

Larsson, L.I., Childers, S. and Synder, S.H. 1979. Met- and leu-enkephalin immunoreactivity in seperate neurones . Nature 282:407-410

Learmonth, J.R. 1931. A contribution to the neurophysiology of the urinary bladder in man. Brain. 54:147-176

Le Greves, P., Nyberg, F., Terenius, L. and Hokfelt, T. 1985. Calcitonin gene-related peptide is a potent inhibitor of substance P degradation. Eur. J. Pharmacol. 115:309-311

Lembeck, F. 1953. Zur frage der zentralen ubertragung afferenter impulse. 111. Das vorkommen und die bedeutung der substanz p in den dorsalen wurzeln des ruckenmarks. Naunyn Schmiedebergs Arch. Pharmakol. 219:197-213

Lepannen, M.K. 1962. A cystometric study of the function of the urinary bladder in prostatic patients. Urol. Int. 14:226-238

Lepor, H., Baumann, M. and Shapiro, E. 1987. Identification and characterization of α1 adrenergic receptors in the canine prostate using [125I]-HEAT. J. Urol. 138:1336-1339

Lepor, H. and Kuhar, M.J. 1984. Characterization and localization of the muscarinic cholinergic receptor in human prostatic tissue. J. Urol. 132:397-402

Lepor, H. and Shapiro, E. 1984. Characterization of α-1 adrenergic receptors in human prostatic hyperplasia. J. Urol. 132:1226-1229

Lepor, H., Shapiro, E., Gup, D. and Baumann, M. 1988. The effect of electrocautery on neurotransmitter receptor binding assays in the canine prostate. J. Urol. 140:668-671

Levin, R.M. and Wein, A.J. 1981. Effect of vasoactive intestinal polypeptide on the contractility of the rabbit urinary bladder. Urol. Res. 9:217-218

Levin, R.M., Jacobowitz, D. and Wein, A.J. 1981. Autonomic innervation of rabbit urinary bladder following estrogen administration. Urol. 17:449-453

Levin, R.M., Staskin, D.R. and Wein, A.J. 1982. The muscarinic cholinergic binding kinetics of the human urinary bladder. Neurourology and Urodynamics. 1:221-226

Levin, R.M., High, J. and Wein, A.J. 1984. The effect of short term obstruction on urinary bladder function in the rabbit. J. Urol. 132:789-791

Levin, R.M., Malkowicz, S.B., Wein, A.J., Atta, M.A. and Elbadawi, A. 1985. Recovery from short-term obstruction of the rat urinary bladder. J. Urol. 134:388-390

Levin, R.M., Memberg, W., Ruggieri, M.R. and Wein, A.J. 1986. Functional effects of in-vitro obstruction on the rabbit urinary bladder. J. Urol. 135:847-851

Levin, R.M., Ruggieri, M.R. and Wein, A.J. 1986. Functional effects of the purinergic innervation of the rabbit urinary bladder. J. Pharmacol. Exp. Ther. 236:452-457

Lowsley, O.S. 1912. The development of the human prostate gland with reference to the development of other structures at the neck of the urinary bladder. Am. J. Anat. 13:299-349

Lundberg, J.M. 1981. Evidence for coexistence of vasoactive intestinal polypeptide (VIP) and acetylcholine in neurons of cat exocrine glands. Morphological, biochemical and functional studies. Acta Physiol. Scand. Suppl. 496:1-57

Lundberg, J.M. and Tatemoto, K. 1982. Pancreatic polypeptide family (APP BPP NPY PYY) in relation to sympathetic vasoconstriction resistant to α-adrenoceptor blockade. Acta Physiol. Scand. 116:393-402

Lundberg, J.M., Torssell, L., Sollevi, A., Pernow, J., Theodorsson Norheim,E., Anggard, A. and Hamberger, B. 1985. Neuropeptide Y sympathetic vascular control in man. Regul. Pept. 13:41-52

Lund, C.J., Benjamin, J.A., Tristan, T.A., Fullerton, R.E., Ramsey, G.H. and Watson, J.S. 1957. Cinefluorographic studies of the bladder and urethra in women. 1. Urethrovesical relationships in voluntary and involuntary urination. Am. J. Obstet. Gynec. 74:896-908

Macdonald, A.D. and McCrea, E.D. 1930. Observations on the control of the bladder. The effects of nervous stimulation and of drugs. Q. J. Exp. Physiol. 20:379-391

Mackenzie, I. and Burnstock, G. 1984. Neuropeptide action on the guinea pig bladder. A comparison with the effects of field stimulation and ATP. Eur. J. Pharmacol. 105:85-94

Maggi, C.A., Santicioli, P., Grimaldi, G. and Meli, A. 1983. The effect of peripherally administered GABA on spontaneous contractions of rat urinary bladder in-vivo. Gen. Pharmacol. 14:455-458

Maggi, C.A., Santicioli, P. and Meli, A. 1984. The effect of capsaicin on micturition reflex in urethrane anaesthetised rats. Proc. Int. Continence Society:217-218

Maggi, C.A., Santicioli, P. and Meli, A. 1985. GABAa and GABAb receptors in detrusor strips from guinea-pig bladder dome. J. Autonom. Pharmacol. 5:55-64

Maggi, C.A. and Meli, A. 1986. The role of neuropeptides in the regulation of the micturition cycle. J. Autonom. Pharmacol. 6:133-162

Maggi, C.A., Barbanti, G., Santicioli, P., Beneforti, P., Misuri, D., Meli, A. and Turini, D. 1989. Cystometric evidence that capsaicin-sensitive nerves modulate the afferent branch of micturition reflex in humans. J. Urol. 142:150-154

Makrigiannis, D. and Gaca, A. 1972. Cystometric studies of detrusor function after prostatectomy andtransurethral electrosurgery. Int. Urol. Nephrol. 4:175-178

Malmgren, A., Sjogren, C., Uvelius, B., Mattiasson, A., Andersson, K.E. and Andersson, P.O. 1987. Cystometrical evaluation of bladder instability in rates with infravesical outflow obstruction. J. Urol. 137:1291-1294

Malmgren, A., Uvelius, B., Andersson, K.E. and Andersson, P.O. 1988. Disappearance of detrusor instability in rats after removal of infravesical outflow obstruction. Neurourology and Urodynamics 7:252-253

Malmgren, A., Uvelius, B., Andersson, K.E. and Andersson, P.O. 1989. On the reversibility of functional bladder changes induced by infravesical outflow obstruction in the rat. J. Urol. (in press)

Malmgren, A., Ekblad, E., Sundler, F., Andersson, K.E. and Andersson, P.O. 1989. Muscarinic supersensitivity in the rat urinary bladder after capsaicin pre-treatment. Unpublished. Personal communication.

Malmgren, A. 1989. On the nature of bladder instability associated with outflow obstruction - an experimental study in the rat. Doctoral Dissertation Lund University, 1-61.

Malone-Lee, J.G. and Chapple, C.R. 1988. A critical review of the development of a software package for clinical urodynamics. in: Medical applications of microcomputers. Coleridge-Smith, P.D. and Scurr, J.H., Eds., Springer-Verlag, London. Berlin. p87-93

Masson, P. 1929. Some histological methods; trichrome staining and their prelimary techniques. Journal of Technical Methods and Bulletin of the International Association of Medical Museums. 12:75-90

Mattiasson, A. and Uvelius, B. 1982. Changes in contractile properties in hypertrophic rat urinary bladder. J. Urol. 128:1340-1342

Mattiasson, A., Andersson, K.E., Sjogren, C., Sundin, T. and Uvelius, B. 1983. Supersensitivity to carbachol in the parasympathetically decentralised feline urinary bladder. Proc. 2nd. Int. Cont. Soc. 77-79

Mattiasson, A., Andersson, K.E. and Sjogren, C. 1984. Adrenoceptors and cholinoceptors controlling noradrenaline release from adrenergic nerves in the urethra of rabbit and man. J. Urol. 131:1190-1195

Mattiasson, A., Andersson, K.E. and Sjogren, C. 1985. Adrenergic and non-adrenergic contraction of isolated urethral muscle from rabbit and man. J. Urol. 133:298-303

Mattiasson, A., Ekblad, E., Sundler, R.F. and Uvelius, B. 1985. Origin and distribution of neuropeptide Y, vasoactive intestinalpolypeptide and substance P containing nerve fibres in the urinary bladder of the rat. Cell Tissue Res. 239:141-146

Mattiasson, A., Andersson, K.E., Elbadawi, A., Morgan, E. and Sjogren, C. 1987. Interaction between adrenergic and cholinergic nerve terminals in the urinary bladder of rabbit, cat and man. J. Urol. 137:1017-1019

Mayo, M.E. and Hinman, F. 1976. Structure and function of the rabbit bladder altered by chronic obstruction or cystitis. Invest. Urol. 14:6-9

McCrea, E.D. 1926. The musculature of the bladder. Proc. R. Soc. Med 19:35-43

McGrath, J.C. 1982. Evidence for more than one type of post-junctional α-adrenoceptor. Biochem. Pharmacol. 31:467-484

McNeal, J.E. 1972. The prostate and prostatic urethra - a morphological synthesis. J. Urol. 107:1008-1016

McNeal, J.E. 1978. Origin and evolution of benign prostatic enlargement. Invest. Urol. 15:340-345

McNeal, J.E. 1980. Anatomy of the prostate; an historical survey of divergent views. The Prostate 1:3-13

Melick, W.F., Naryka, J.J. and Schmidt, J.H. 1961. Experimental studies of urethral peristaltic patterns of the pig: 1. Similarity of pig and human ureter and bladder physiology. J. Urol. 85:145-148

Mettler, C.C. 1947. History of Medicine. Mettler, F.A. Ed. Blackiston Company.

Meyhoff, H.H., Nordling, J. and Hald, T. 1984. Urodynamic evaluation of transurethral verses transvesical prostatectomy. Scand. J. Urol. 18:27-35

Millar, A. 1958. The aetiology and treatment of diverticulum of the bladder. Br. J. Urol. 30:43-56

Miller, H.G., Simpson, C.A. and Yeates, W.K. 1965. Bladder dysfunction in multiple sclerosis. Br. Med. J. 1:1265-1269

Miller, R.J. 1981. Peptides as neurotransmitters focus on the enkephalins and endorphins. Pharmacology and therapeutics 12:73-108

Mobley, T.L., Elbadawi, A., McDonald, D.F. and Schenk, E.A. 1966. Innervation of the human urinary bladder. Surgical Forum. 27:505-506

Moore, R.A. 1935. The morphology of small prostatic carcinoma. J. Urol. 33:224-234

Moore, T.D. 1924. Bladder sensibility. Arch. Surg. 9:176-187

Morgagni, G. 1769. The seats and causes of disease. in: Investigated by Anatomy Book 3. Miller, A. and Cadell, T., Eds., London, Johnson & Payne, 46-462

Mori, J. 1955. Histology and innervation of prostate and pars pelocina urethrae in cat. Arch. Hist. Jap. 8:227-241

Mosso, A. and Pellacani, P. 1882. Sur les fonctions de la vessie. Arch. Ital. Biol. 1:97-128, 291-324

Moulds, R.W. and Jauerning, R.A. 1977. Mechanism of prazosin collapse. Lancet. 1:200-201

Moyer, T.P. and Jiang, N.S. 1978. Optimised isocratic conditions for analysis of the catecholoamines by high performance reversed-phase paired ion chromatography with amperometric detection. J. Chromatogr. 153:153-165

Mulderry, P.K., Ghatei, M.A., Rodrigo, J., Allen, J.M., Rosenfeld, M.G., Polak, J.M. and Bloom, S.R. 1985. CGRP in cardiovascular tissues of the rat. Neurosci. 14:947-954

Mundy, A.R. 1984. Neuropeptides in lower urinary tract function. World J. Urol. 2:211-215

Murphy, L.J.T. 1972. The history of urology. Illinois, Thomas.

Mutoh, S., Ueda, S., Fukumoto, Y., Machida, J. and Ikegami, K. 1987. Effect of adrenergic and cholinergic drugs on the noradrenergic transmission in bladder neck smooth muscle. J. Urol. 138:212-215

Nathan, P.W. and Smith, M.C. 1951. The centripetal pathway from the bladder and urethra within the spinal cord. J. Neurol. Neurosurg. Psychiat. 14:262-280

Nathan, P.W. 1956. Sensations associated with micturition. Br. J. Urol. 28:126-131

Nergardh, A. and Boreus, L.O. 1972. Autonomic receptor function in the lower urinary tract of man and cat. Scand. J. Urol. Nephrol. 6:32-36

Nergardh, A. and Boreus, L.O. 1973. The functional role of cholinergic receptors in the outlet region of the urinary bladder; an in-vitro study in the cat. Acta Pharmacol. et Toxicol. 32:467-480

Nergardh, A. 1975. Autonomic receptor functions in the lower urinary tract: a survey of recent experimental results. J. Urol. 113:180-185

Nergardh, A., Boreus, L.O. and Naglo, A.S. 1977. Characterisation of the adrenergic β receptor in the urinary bladder of man and cat. Acta Pharmacol. Toxicol. 40:14-21

Nergardh, A. and Kinn, A.C. 1983. Neurotransmission in activation of the contractile response in the human urinary bladder. Scand. J. Urol. Nephrol. 17:135-137

Nicoll, R.A., Schenker, C. and Leeman, S.E. 1980. Substance P as a transmitter candidate. Am. Rev. Neuro. Sci. 3:227-268

Nilvebrant, L., Andersson, K.E. and Mattiasson, A. 1985. Characterisation of the muscarinic cholinoceptors in the human detrusor. J. Urol. 134:418-423

Nilvebrant, L. 1986. On the muscarinic receptors in the urinary bladder and the putative subclassification of muscarinic receptors. Acta. Pharm. Toxicol. 59:Suppl 1, 1-45

Nishizawa, O., Fukuda, T., Matsuzaki, A., Moriya, I., Harada, T. and Tsuchida, S. 1985. The role of the sympathetic nerves in bladder and urethral sphincter function during the micturition cycle in the dog evaluated by pressure flow EMG study. J. Urol. 134:1259-1261

Nordling, J. 1983. Influence of the sympathetic nervous system on lower urinary tract in man. Neurourology and Urodynamics. 2:3-26

Norlen, L. 1977. Effects on the urinary bladder and urethra of different pharmacological treatments. An in-vitro study in normal and parasympathetically denervated cats. Scand. J. Urol. Nephrol. 11:7-16

Norlen, L., Sundin, T. and Waagstein, F. 1978, β-adrenoceptor stimulation of the human urinary bladder in-vitro. Acta Pharmacol. toxicol.(suppl) 43:26-30

North, R.A., Katayama, Y. and William, J.T. 1979. On the mechanism and site of action of enkephalin on single myenteric neurones. Brain Res. 165:67-77

O'Malley, C.D., Saunders, J.B., De C M 1952. Eds. Leonardo da Vinci on the Human Body (genitourinary system).

Onufrowicz, B. 1900. On the arrangements and function of the cell groups of the sacral region of the spinal cord in man. Arch. Neurol. Psychopathol. 3:387-412

Perlberg, S. and Caine, M. 1982. Adrenergic response of bladder muscle in prostatic obstruction. Urology 20:524-527

Pittam, B.S., Burnstock, G. and Purves, R.D. 1987. Urinary bladder intramural neurones an electrophysiological study utilising a tissue culture preparation. Brain Res. 403:267-278

Polak, J.M. and Bloom, S.R. 1984. Localisation and measurement of VIP in the genitourinary system of man and animals. Peptides. 5:225-230

Porter, N.H. 1962. A physiological study of the pelvic floor in rectal prolapse. Ann. R. Coll. Surg. 31:379-404

Powell, P.H and Fenely, R.C.L. 1980. The role of urethral sensation in clinical urology,. Br. J. Urol. 52:539-541

Price, D.A., Ramsden, P.D. and Stobbart, D. 1980. The unstable bladder and prostatectomy. Br. J. Urol. 52:529-531

Raezer, D.M., Wein, J., Jacobwitz, D. and Corriere, J.N.J. 1973. Autonomic innervation of canine urinary bladder. Cholinergic and adrenergic contributions and interaction of sympathetic and parasympathetic nervous systems in bladder function. Urology 2:211-214

Raz, S. 1983. Peripheral bladder denervation. Proc. 2nd. Int. Cont. Soc. :177-180

Reuther, K., Aagaard, J. and Sander Jensen, K. 1983. Lignocaine test and detrusor instability. Br. J. Urol. 55:493-494

Reuther, K. and Aagaard, J. 1984. Alpha-adrenergic blockade in the diagnosis of detrusor instability secondary to intravesical obstruction. Urol. Int. 39:312-313

Robinson, P.P. 1971. The demonstration of acetylcholinesterase in autonomic axons with the electron microscope. Prog. Brain Res. 34:357-361

Rohner, T.J., Hannigan, J.D. and Sanford, E.J. 1978. Altered in-vitro adrenergic responses of dog detrusor muscle after chronic bladder outlet obstruction. Urology. 11:357-361

Rose, D.K. 1932. Changes in the wall of the bladder secondary to prostatic obstruction. Arch. Surg. 25:783-795

Said, S.I. and Mutt, V. 1970. Polypeptide with broad biological activity. Isolation from small intestine. Science. 169:1217-1218

Said, S. 1984. Vasoactive intestinal polypeptide (VIP) current status. Peptides. 5:143-150

Santicioli, P., Maggi, C.A. and Meli, A. 1985. The effect of capsaicin pretreatment on the cystometrograms of urethrane anaesthetised rats. J. Urol. 133:700-703

Schultzberg, M., Hokfelt, T., Nilsson, L., Rehfeld, J.F., Brown, M., Elde, R., Goldstein, M. and Said, S. 1980. Distribution of peptide and catecholamine containing containing neurons in the gastrointestinal tract of rat and guineapig; Immunohistochemical studies with antisera to substance P, VIP, Enk, somatostatin, gastrin, CCK, neurotensin, DBH. Neuroscience. 5:689-744

Sehn, J.T. 1978. Anatomic effect of distension therapy in unstable bladder: new approach. Urology. 11:581-587

Sehn, J.T. 1979. The ultrastructural effect of distension on the neuromuscular apparatus of the urinary bladder. Invest. Urol. 16:369-375

Sethia, K.K. and Smith, J.C. 1987. The effect of pH and ligocaine on detrusor instability. Br. J. Urol. 60:516-518

Seto, H. 1954. Histological studies on the sensory terminations distributed in the circulatory system and the urogenital organs . Arch. Hist. Jap. 6:665-678

Shapiro, A., Mazouz, B. and Caine, M. 1981. The α adrenergic effect of prazosin on the human prostate. Urol. Res. 9:17-20

Shapiro, E. and Lepor, H. 1986. Alpha-2 adrenergic receptors in hyperplastic human prostate: identification and characterisation using (3H)Rauwolscine. J. Urol. 135:1038-1042

Shapiro, E., Tsitlik, J.E. and Lepor, H. 1987. Alpha-2 adrenergic receptors in canine prostate: biochemical and functional correlations. J. Urol. 137:565-570

Sharkey, K.A., Williams, R.G., Schultzberg, M. and Dockray, G. 1983. Sensory substance P innervation of the urinary bladder possible site of action of capsaicin in causing urinary retention in rats. Neuroscience. 10:861-868

Shelley, H.S. 1965. The enlarged prostate, a brief history of its treatment. Journal of the History of Medicine. 20:452-473

Shigematsu, H. 1928. Etude experimentale de la retention d'urine. J. Urol. (Paris) 25:16-21

Shirai, M., Sasaki, K. and Rikimaru, A. 1973. A histochemical investigation of the distribution of adrenergic and cholinergic nerves in the human male genital organs. Tohoku J. Exp. Med. 111:281-291

Sibley, G.N.A. 1984a. A comparison of spontaneous and nerve mediated activity in bladder muscle from man, pig and rabbit. J. Physiol. (Lond) 354:431-443

Sibley, G.N.A. 1984b. The response of the bladder to lower urinary tract obstruction. D M Thesis. University of Oxford.

Sibley, G.N.A. 1985. An experimental model of detrusor instability in the obstructed pig. Br. J. Urol. 57:292-298

Sibley, G.N.A. 1987. The physiological response of the detrusor muscle to experimental bladder outflow obstruction in the pig. Br. J. Urol. 60:332-336

Simonds, W.F., Booth, A.M., Thor, K.B., Ostrowski, N.L., Nagel, J.R. and De Groat, W.C. 1983. Parasympathetic ganglia naloxone antagonises inhibition by leucine-enkephalin and GABA. Brain Research. 271:365-370

Sjogren, C., Andersson, K.E., Husted, S., Mattiasson, A. and Moller-Madsen, B. 1982. Atropine resistance of transmurally stimulated isolated human bladder muscle. J. Urol. 128:1368-1371

Sjogren, C., Andersson, K.E. and Mattiasson, A. 1985. Effects of vasoactive intestinal polypeptide on isolated urethral and urinary bladder smooth muscle from rabbit and man. J. Urol. 133:136-140

Smith, E.R., Ilievski, V. and Hadidian, Z. 1966. The stimulation of canine prostatic secretion by pilocarpine. J. Pharmacol. Exp. Ther. 151:59-65

Smith, J.C. 1968. Urethral resistance to micturition. Br. J. Urol. 40:125-156

Smith, J.C. 1976. The function of the bladder. In Urology. Vol 2. Blandy, J., Ed. Blackwell, Oxford. p672-686

Speakman, M.J., Brading, A.F., Gilpin, C.J., Dixon, J.S., Gilpin, S.A. and Gosling, J.A. 1987. Bladder outflow obstruction - a cause of denervation supersesitivity. J. Urol 138:1461-1466

Speakman, M. J., Walmsley, D. and Brading, A.F. 1988. An in vitro pharmacological study of the human trigone - a site of non-adrenergic, non-cholinergic neurotransmission. Br. J. Urol. 61:304-309

Steers, W.D. and de Groat, W.C. 1988. Effect of bladder outlet obstruction on micturition reflex pathways in the rat. J. Urol. 140:864-871

Steers, W.D., Ciambotti, J.A., Etzel, B., Erdman, B., Erdman, S. and De Groat, W.C. 1989. Morphological changes in afferent and efferent pathways to the urinary bladder following urethral obstruction in the rat. J. Urol. 141:323A

Stjernschantz, J., Gregerson, D., Bauscher, L. and Sears, M. 1982. Enzyme-linked immunorsorbent assay of sutstance P; a study in the eye. J. Neurochem. 38:1323-1328

Stockamp, K. and Schreiter, F. 1974. Function of the posterior urethra in ejaculation and its importance for urine control. Urol. Int. 29:226-230

Sundin, T. and Dahlstrom, A. 1973. The sympathetic innervation of the urinary bladder in the normal state and after parasympathetic denervation at the spinal root level. Scand. J. Urol. Nephrol. 7:131-149

Sundin, T., Dahlstrom, A., Norlen, L. and Svedmyr, N. 1977. The sympathetic innervation and adrenoceptor function of the human lower urinary tract in the normal state and after parasympathetic denervation. Invest. Urol. 14:332-328

Sundler, F., Brodin, E., Ekblad, E., Hakansson, R. and Uddman, R. 1985. Sensory nerve fibres distribution of substance P, neurokinin A and calcitonin gene-related peptide In Tachykinin antagonists. Hakansson, R., and Sundler, F., Eds. Amsterdam, Elsevier, p3-14

Susset, J.G., Serlot-Viguier, D., Lamy, F., Madernas, P. and Black, R. 1978. Collagen in 155 human bladders. Invest. Urol. 16:204-206

Szolesanyi, J. 1977. A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J. Physiol. 73:251-259

Taira, N. 1972. The autonomic pharmacology of the bladder. Ann. Rev. Pharmacol. 12:197-208

Tanagho, E.A.and Pugh, R.C.B. 1963. The anatomy and function of the ureterovesical junction. J. Urol. 35:151-165

Tanagho, E.A., Hutch, J.A., Meyers, F.H. and Rambo, O.N. 1965. Primary vesicoureteral reflux: experimental studies of its aetiology. J. Urol. 93:165-176

Tanagho, E.A. and Smith, D.R. 1966. The anatomy and function of the bladder neck. Br. J. Urol. 38:54-71

Tanagho, E.A. and Miller, E.R. 1970. Initiation of voiding. Br. J. Urol. 42:175-183

Tang, P.C. and Ruch, T.C. 1955. Non-neurogenic basis of bladder tonus. Am. J. Physiol. 181:249-257

Thompson, H. 1882. The diseases of the prostate; their pathology and treatment. London, J & A Churchill. p1-152

Timmermans, P.B.M.W.M. and Van Zwieten, P.A. 1981. Mini-review. The postsynaptic α-2 adrenoceptor. J. Auton. Pharmacol. 1:171-183

Tisell, L.E. and Salander, H. 1975. The lobes of the human prostate. Scand. J. Urol. Nephrol. 9:185-191

Todd, J.K. and Mack, A.J. 1969. A study of human bladder detrusor muscle. Br. J. Urol. 41:448-454

Trendelenburg, U. 1963. Supersensitivity and subsensitivity to sympathomimetic amines. Pharmac. Rev. 18:629-640

Turner-Warwick, R.T. 1971. The Urodynamic Clinic - clinical problems associated with urodynamic abnormalities with special reference to the clinical importance of detrusor function studies. First International Symposium for Urodynamics. London, Springer-Verlag.

Turner-Warwick, R.T., Whiteside, C.G., Arnold, E.P., Bates, C.P., Worth, P.H.L., Milroy, E.J.M., Webster, J.R. and Weir, J. 1973. A urodynamic view of prostate obstruction and the results of prostatectomy. Br. J. Urol. 45:632-645

Turner-Warwick, R.T. 1984. Bladder outflow obstruction in the male. In, Urodynamics - Principles Practice and Application. Mundy, A.R., Stephenson, T.P. and Wein, A.J. Eds., Churchill Livingstone, London. p183-204

Uemura, E., Fletcher, T.F. and Bradley, W.E. 1975. Distribution of lumbar and sacral aferent axons in submucosa of cat urinary bladder. Anat. Rec. 183:579-588

Uvelius, B., Persson, L. and Mattiasson, A. 1984. Smooth muscle cell hypertrophy and hyperplasia in the rat detrusor after short-term infravesical outflow obstruction. J. Urol. 131:173-176

Uvelius, B., Arner, A. and Malmquist, U. 1988. Influence of hypoxia on metabolic rates and contraction in urinary bladder from rats with infravesical outflow obstruction. Neurourology and Urodynamics. 7:245-246

Uvelius, B., Arner, A. and Malmquist, U. 1989. Contractile and cytoskeletal proteins in detrusor muscle from obstructed rat and human bladder. Neurourology and Urodynamics. 8:396-398

Vaalasti, A. and Hervonen, A. 1979. Innervation of the ventral prostate of the rat. Am. J. Anat. 154:231-244

Vaalasti, A. and Hervonen, A. 1980a. Autonomic innervation of the human prostate. Invest. Urol. 17:293-297

Vaalasti, A. and Hervonen, A. 1980b. Nerve endings in the human prostate. Am. J. Anat. 157:41-47

Vaalasti, A., Linnoila, I. and Hervonen, A. 1980c. Immunohistochemical demonstration of VIP and enkephalin immunoreactive nerve fibres in the human prostate and seminal vesicles. Histochemistry. 66:89-98

Vaalasti, A., Tainioh, H., Pelto-Huikko, M. and Hervonen, A. 1986. Light and electron microscope demonstration of VIP- and enkephalin- immunoreactive nerves in the human male genitourinary tract. Anat. Rec. 215:21-27

Van Brummelen, P., Vermey, P., Timmermans, P.B.M.W.M. and Van Zwieten, P.A. 1982. Prelimary evidence for a postsynaptic α-2 adrenoceptor in the vasculature of the human forearm. Br. J. Pharmacol. :134P

Vanov, S. 1965. Responses of the rat urinary bladder in situ to drugs and nerve stimulation. Br. J. Pharmacol. 24:591-600

Vodusek, D.B. and Light, J.K. 1983. The motor nerve supply of the external urethral sphincter muscles; an electrophysiological study. Neurourology and Urodynamics. 2:193-200

Von Euler, U.S. and Gaddum, J.H. 1931. An unidentified depressor substance in certain tissue extracts. J. Physiol. (Lond) 72:74-87

Von Garrelts, B. 1956. Analysis of micturition. A new method of recording the voiding of the bladder. Acta Chir. Scand. 112:326-340

Walker. J. and Bates, C.P. 1985. Responses of isolated strips of smooth muscle from the human bladder neck. Proc. 15th Int. Cont. Soc. :168-169

Warwick, R. and Williams, P.L. 1973. Neurology -plexuses of the autonomic nervous system. in: Gray's Anatomy, Warwick, R. and Williams, P.L., Eds., Edinburgh, Longman, p1079-1081

Wein, A.J., Gregory, J.G., Cromie, W.J., Corriere, J.N.J. and Jacobwitz, D. 1974. Sympathetic innervation and chemical sympathectomy of canine bladder. Urology. 9:27-31

Wein, A.J. and Levin, R.M. 1979. Comparison of adrenergic receptor density in urinary bladder in man, dog and rabbit. Surgical Forum. 30:576-578

Wesson, M.B. 1920. Anatomical, embryological and physiological studies of the trigone and neck of the bladder. J. Urol. 4:279-307

Westfall, D.P. 1981, Supersensitivity of smooth muscle in smooth muscle:. An assessment of current knowledge. Bulbring, E., Brading, A.F., Jones, A.W. and Tomitat, T. Eds., Arnold, London.

Williams, M.A. 1977. Quantitative methods in biology. in: Practical methods in electron microscopy. Glauert, A.M., Ed., Amsterdam, North Holland, Biomedical press vol 6 part 2, p33-37

Winter, D.L. 1971. Receptor characteristics and conduction velocities in bladder afferents. J. Psychiat. Res. 8:225-235

Woodburne, R.T. 1960. Structure and function of the urinary bladder. J. Urol. 84:79-85

Yokokawa, K.M., Tohuama, M., Shiosaka, S., Shiotani, Y., Sonoda, T., Emson, P., Hillyard, V., Girgis, S. and Macintyre, I. 1986. Distribution of calcitonin gene-related peptide containing fibres in the urinary bladder of the rat and their origin. Cell Tissue Res. 244:271-278

Young, H.H. and Wesson, M.B. 1921. The anatomy and surgery of the trigone. Arch. Surg. 3:1-37

Young, W.S. and Kuhar, M.J. 1979. A new method for receptor autoradiography: (3H) opioid receptors in rat brain. Brain Res. 179:255-270