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Urinary Bladder Contraction and Relaxation: Physiology and Pathophysiology

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Andersson, Karl-Erik, and Anders Arner. Urinary Bladder Contraction and Relaxation: Physiology and Pathophysiology. *Physiol Rev* 84: 935–986, 2004; 10.1152/physrev.00038.2003.—The detrusor smooth muscle is the main muscle component of the urinary bladder wall. Its ability to contract over a large length interval and to relax determines the bladder function during filling and micturition. These processes are regulated by several external nervous and hormonal control systems, and the detrusor contains multiple receptors and signaling pathways. Functional changes of the detrusor can be found in several clinically important conditions, e.g., lower urinary tract symptoms (LUTS) and bladder outlet obstruction. The aim of this review is to summarize and synthesize basic information and recent advances in the understanding of the properties of the detrusor smooth muscle, its contractile system, cellular signaling, membrane properties, and cellular receptors. Alterations in these systems in pathological conditions of the bladder wall are described, and some areas for future research are suggested.

I. INTRODUCTION

The urinary bladder has two important functions: storage of urine and emptying. Storage of urine occurs at low pressure, which implies that the bladder relaxes during the filling phase. Disturbances of the storage function may result in lower urinary tract symptoms (LUTS), such as urgency, frequency, and urge incontinence, the components of the overactive bladder syndrome (3). The overactive bladder syndrome, which may be due to involuntary contractions of the smooth muscle of the bladder (detrusor) during the storage phase, is a common and underreported problem, the prevalence of which has only recently been assessed (467).

Emptying requires a coordinated contraction of the bladder and relaxation of the urethra. Disturbances of the

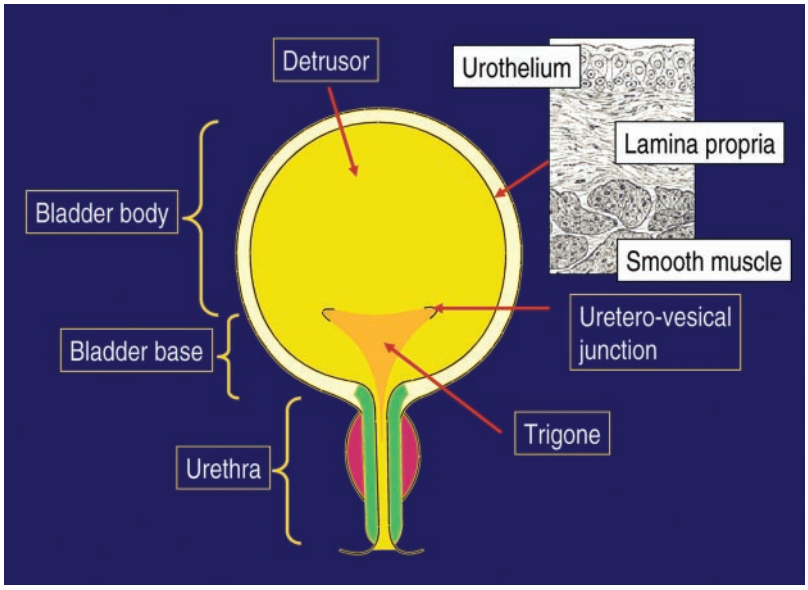

FIG. 1. Schematic drawing of the bladder.

voiding function can lead to symptoms of hesitancy, weak stream, feeling of incomplete bladder emptying, and postmicturition dribble. LUTS is increasing markedly with age in both males and females and is a major problem in the elderly population. Partly due to an increasing awareness of the problem of LUTS, and the lack of effective treatment of the disorder, interest in the research of lower urinary tract physiology and pathophysiology has increased.

To find ways to control micturition, knowledge about the mechanisms of contraction and relaxation of detrusor smooth muscle under normal and pathological conditions is necessary. It is well known that smooth muscles exhibit an extreme variability, not only in ultrastructural details, but also in their contractile, regulatory, and electrophysiological properties and in their sensitivities to drugs and neurotransmitters. Nevertheless, most smooth muscles have properties in common. This fact may be of interest because therapeutic approaches based on findings in other types of smooth muscle may be applied also to those of the lower urinary tract.

To identify unique properties of the muscles of the lower urinary tract, possibly involved in pathological conditions, or as potential targets for therapeutic interventions, is a challenge to the research field. Much of our current knowledge is based on animal experimentation, and species differences may be a problem when extrapolating animal findings to the human situation.

Many factors, e.g., central and peripheral nervous control and the contribution of other components of the lower urinary tract, may influence micturition. In the last decade many reviews have focused on different aspects of the physiology and pathophysiology of the bladder (18–20, 147, 174, 203, 253, 325, 478, 506, 666, 675, 697).

The present overview is focused on the processes, from cellular receptors to the contractile machinery, involved in physiological contraction and relaxation of bladder smooth muscle (detrusor). Special attention has been given to the role of these processes in pathophysiological alterations in bladder function associated with, e.g., bladder outlet obstruction, detrusor hypertrophy, and detrusor overactivty. The size of the bladder varies over a large range between species (bladder capacity: mouse, ~0.15 ml; rat, ~1 ml; human, ~500 ml). Also micturition patterns, contractile properties, and contractile regulation vary between species. When possible in this review, information on human detrusor is discussed. Because in several respects basic information on the properties of the human detrusor is fragmentary or missing, animal data will be presented, when appropriate.

II. MORPHOLOGY OF THE LOWER URINARY TRACT

The lower urinary tract consists of the urinary bladder and the urethra (Fig. 1). The urethra contains both smooth and striated muscles, and details on its structure can be found elsewhere (76, 717). The bladder can be divided into two main components: the bladder body, which is located above the ureteral orifices, and the base, consisting of the trigone, urethrovesical junction, deep detrusor, and the anterior bladder wall. The bladder is a hollow smooth muscle organ lined by a mucous membrane and covered on its outer aspect partly by peritoneal serosa and partly by fascia. Its muscular wall is formed of smooth muscle cells, which comprise the detrusor muscle. The detrusor is structurally and functionally different from, e.g., trigonal and urethral smooth muscle. As

pointed out above, in this review, focus is on properties of the detrusor smooth muscle.

Details on the morphology of the detrusor can be found in many reviews and textbooks (148, 152, 173, 214). Thus only a few aspects are discussed here. Three layers of smooth muscle have been described. The cells of the outer and inner layers tend to be oriented longitudinally, and those of the middle layer circularly. In the human detrusor, bundles of muscle cells of varying size are surrounded by connective tissue rich in collagen. These bundles vary extensively in size. In the human detrusor they are large, often a few millimeters in diameter, and composed of several smaller sub-bundles. The bundles are not clearly arranged in distinct layers, but run in all directions. Cells with long dendritic processes can be found parallel to the smooth muscle fibers. These cells contain vimentin, an intermediate filament protein expressed by cells of mesenchymal origin and nonmuscle myosin (cf. Refs. 158, 408, 599, 607 and sect. v*G*). The functional importance of these cells has not been established.

Within the main bundles, the smooth muscle cells may exist in groups of small functional units, or fascicles (see Ref. 159). The orientation and interaction between the smooth muscle cells in the bladder are important, since this will determine how the bladder wall behaves and what effect activity in the cells will have on its shape and intraluminal pressure. In smaller animals, e.g., rabbit, the muscle bundles are less complex and the patterns of arrangement simpler than in the human detrusor.

The individual smooth muscle cells in the detrusor are typical smooth muscle cells, similar to those in other muscular organs. They are long, spindle-shaped cells with a central nucleus. When fully relaxed, the cells are several hundred microns long, and the widest diameter is 5–6 μm. The cytoplasm is packed with the normal myofilaments, and the membranes contain regularly spaced dense bands, with membrane vesicles (caveoli) between them. There are also scattered dense bodies in the cytoplasm. Mitochondria and fairly sparse elements of sarcoplasmic reticulum (mostly near the nucleus) are also present (152).

III. THE CONTRACTILE SYSTEM

A. Key Features of the Detrusor Smooth Muscle Cells

The two main functions of the lower urinary tract, to store urine without leakage for longer periods of time and to rapidly expel it during micturition, occur naturally in normal life. They involve a very complex interaction between the structural/anatomic parts of the urinary tract and between nervous control systems. In addition to these demands on integrative control, both filling and emptying of the urinary bladder provide a challenge to the muscle components in the walls of the lower urinary tract. During filling of the urinary bladder, the smooth muscle cells have to relax, and to elongate and rearrange in the wall over a very large length interval. During micturition, force generation and shortening must be initiated comparatively fast, be synchronized, and occur over a large length range. These activities thus require both regulation of contraction and regulation of relaxation. To respond to the nervous and hormonal control systems, each part of the urinary tract muscles has to have specific receptors for the transmitters/modulators, released from nerves or generated locally, and the associated cellular pathways for initiating contraction and relaxation.

In early work by Emil Bozler (71), two classes, "single-unit" and "multiunit," of smooth muscle were defined on the basis of contractile behavior. Bozler's class of single-unit smooth muscles is described to be arranged in sheets or bundles, and the cell membranes have many points of close contact, gap junctions. Gap junctions constitute low-resistance pathways, formed by connexin subunits, through which ions can flow from one cell to the other, and thereby an electrical signal can be spread rapidly throughout the tissue (cf. Ref. 82). Action potentials can thus be conducted from one area to another by direct electrical conduction. A minority of the fibers in a single-unit muscle spontaneously generates action potentials, pacemaker cells. Characteristically, in single-unit smooth muscles a contractile response can often be induced by stretching the muscle. Multiunit smooth muscle are thought to be composed of discrete muscle fibers or bundles of fibers that operate independently of each other. They are richly innervated by the autonomic nervous system and are controlled mainly by nerve signals. They rarely show spontaneous contractions. In practice, multiunit muscles would be very rare since the vast majority of smooth muscles have some degree of interconnection between cells. However, although the detrusor muscle exhibits several of the characteristics ascribed to a single-unit smooth muscle, it also shows several features ascribed to multiunit smooth muscles, being densly innervated, and functionally requiring nervous coordination to achieve voiding.

An alternative division of smooth muscles into "phasic" and "tonic" smooth muscle has been proposed (613). This division into these types is based on membrane properties and contractile behavior. As shown by Horiuti et al. (285), the phasic and tonic smooth muscle types have different kinetics of both the regulatory systems and of the contractile machinery. The contractile kinetics of different smooth muscles most likely reflect a continuum, and division into a fast phasic type and a slow tonic type might be difficult. As discussed below, the urinary bladder smooth muscle is a comparatively fast smooth muscle with characteristics of a "phasic" smooth muscle.

B. Structure of the Contractile Apparatus and Cytoskeleton

1. Contractile proteins and filaments

Contraction of smooth muscle is due to interaction between the contractile proteins actin and myosin in a similar manner as in other muscle types. Regular sarcomeric structures are lacking in smooth muscle, and the structure of the thin (actin-containing) and the thick (myosin-containing) filaments and their cellular organization in smooth muscle are at present not fully characterized. Recently, a large, megaDalton, structural protein, smitin, has been described to be present in smooth muscle and to interact with smooth muscle myosin filaments (344), which could suggest that large titinlike proteins are involved in the arrangement of the smooth muscle contractile apparatus. Cytoplasmic dense bodies, which contain α-actinin, have been found to be mechanically connected and suggested to provide a framework for the attachment of the contractile structures (66, 223, 324, 665). The organization and length, or length distribution, of such "sarcomere equivalents" or "contractile units" in smooth muscle would be an important factor determining the force and shortening velocity of smooth muscle and the length interval over which a smooth muscle can generate force. The length of the contractile unit would be dependent on the length of the contractile filaments. The actin filament length has not been unequivocally determined in smooth muscle. Values similar to those in striated muscle have been presented, as well as higher values (160, 606). The thick filament length has been reported to be slightly longer in smooth (2.2 μm; Ref. 44) compared with skeletal muscle ($\sim$1.6 μm). At present, no unique structural features of the contractile unit in the urinary bladder have been associated with the large volume and cell length span over which the urinary bladder muscle can operate. The arrangement of contractile filaments appears to be generally similar to that of other types of smooth muscle (153, 214). Longer contractile units could theoretically give a longer length interval over which the cells could contract. However, this would also lead to a lower maximal shortening velocity (562). Because the urinary bladder is a comparatively fast smooth muscle with a maximal shortening velocity similar to that of other smooth muscles with similar contractile protein isoform composition, the contractile units do not seem to be extensively long in the urinary bladder. The changes in bladder volume are directly coupled with changes in cell length (671, 677), also excluding sliding or rearrangement of the smooth muscle cells in the tissue during bladder filling and emptying.

The actin concentration in smooth muscle is similar to that of skeletal muscle and has been reported to be in the range of 20–50 mg/g smooth muscle cell for vascular tissue (125, 436, 486, 487). The value determined for rat and mouse urinary bladder, $\sim$40 mg/g smooth muscle cell, falls within this range (440, 598). Four different isoforms of actin are expressed in smooth muscle, α-, β-, and two forms of γ-actin (681). In bladder muscle, α-, β-, and γ-actin (comprising both smooth and nonmuscle γ-actin) are present in the relative proportions 33:25:42% for human (441), 41:19:40% for rat (440), and 44:10:46% for mouse (456). The functional roles of the actin isoforms and the consequences of isoform shifts are unclear at present. The cellular distribution of the actin isoforms has been reported to differ, β-actin is predominantly found in the cytoskeletal domain of some smooth muscle cells (508), and it is possible that the different actin isoforms have different functions in the cytoskeleton. With regard to the contractile properties, it should be noted that actin is a highly conserved protein and the different isoforms have a significant degree of homology (549). The filament translocation velocity in the in vitro motility assay was similar under a variety of conditions for smooth and skeletal actin (259). Smooth muscle preparations with different isoactin composition appear functionally similar (160). It is thus unlikely that the actin isoform composition is a major factor determining the extent of force development or the shortening velocity of smooth muscle.

The amount of myosin in smooth muscle is approximately three to five times less than in skeletal muscle (10–16 mg/g smooth muscle cell, Refs. 436, 487) and the ratio of actin to myosin filaments is $\sim$15:1, compared with 2:1 in striated muscle (cf. Ref. 618). In bladder tissue, the myosin amount is in the range above (rat, 17 mg/g smooth muscle cell, Ref. 440; mouse, 26 mg/g smooth muscle cell, Ref. 598). Myosin molecules polymerize with the globular head regions, containing the nucleotide and actin binding sites, projecting at regular $\sim$14-nm intervals to form cross-bridges with actin. The detailed assembly of the smooth muscle thick filament is not resolved; both phase- or side-polar filaments with cross-bridges projecting in an antiparallel fashion on both sides of the filament (127, 133, 603, 730) and bipolar filaments, with a bare zone and with cross-bridges projecting in opposite directions at each end like in skeletal muscle thick filaments (44), have been proposed.

Smooth muscle myosin belongs to the myosin II superfamily of filament forming myosin motors (cf. Ref. 584). The myosin molecule is a hexamer formed by six polypeptide chains: two heavy chains and two pairs of light chains. The carboxy-terminal parts of the heavy chains dimerize into an α-helical coiled tail region constituting the backbone of the thick filaments. Two light chains, one essential (molecular mass 17 kDa) and one regulatory (20 kDa), are associated with each of the two amino-terminal head parts of the heavy chains (cf. Ref. 5).

The smooth muscle myosin heavy chain (SM-MHC) is encoded by a single gene (491, 737). Different myosin

heavy chain isoforms can be formed by alternative splicing. With the use of gel electrophoresis (163, 561), two smooth muscle two heavy chain variants, the SM1 (molecular mass ~204 kDa) and the SM2 (~200 kDa), have been identified. These two isoforms are formed by alternative splicing (47, 490), where the larger SM1 isoform contains a unique sequence of 43 amino acids in the carboxy-terminal tail region comprising a phosphorylation site for casein kinase II (330, 332, 333) and the smaller SM2 isoform 9 unique amino acids. The relative expression of SM1 and SM2 varies between adult smooth muscle tissues and between cells in the tissue (462, 463, 569, 586). During fetal life the expression of SM1 is high and the ratio SM1/SM2 decreases with development (30, 31, 164, 165, 470). In fetal and neonatal bladders, SM1 is the predominant isoform (30, 400). In the adult urinary bladder the relative content of SM1 is ~70% of the myosin heavy chain in the rat (440) and 40% in the rabbit (30). No major differences have been noted between the bladder parts (292). In urinary bladder of humans, the SM1 content is ~40% (193, 441).

The functional consequences of changes in the SM1/SM2 ratio in smooth muscle tissue is unclear. A few studies have reported a correlation between tissue SM1/SM2 expression and the maximal shortening velocity, as an index of the kinetics of the actin-myosin interaction (280). However, it should be noted that the SM1 and SM2 differ in the tail region, which does not primarily interact with actin and other studies using in vitro motility assay, isolated cells or a comparative approach (334, 437, 463, 475, 586) have failed to show a correlation between shortening velocity and SM1/SM2 ratio. A correlation between SM1/SM2 ratio and the extent of cell shortening has been reported (462). The SM1 and SM2 exhibit differences in their filament assembly properties (559), and the SM1 contains a phosphorylation site in the tail region, which could suggest that the expression of these isoforms can be important for the structure of the contractile machinery.

Two additional myosin heavy chain isoforms, SM-A and SM-B, are formed by alternative splicing in the amino-terminal region. The SM-B has an extra seven-amino acid insert in the loop 1 region of myosin close to the ATPase site, coded by exon 5B (46, 48, 254, 335, 713). Both the SM1 and the SM2 can contain the insert, enabling four possible isoforms SM1-A, SM1-B, SM2-A, and SM2-B (cf. Ref. 49). The insert in loop 1 region at the 25/50-kDa domain junction in the head of the myosin heavy chain is close to the catalytic site and has been suggested to modulate actin-myosin kinetics (633). It was early recognized that this region of myosin influences the kinetics of its interaction with actin. With the use of the in vitro motility assay (335, 377, 560), it was shown that myosin with the insert (SM-B) propels actin at a higher velocity than myosin without. The relative SM-B expression varies between tissues, and comparative studies on muscle preparations have found a correlation between expression of the inserted (SM-B) isoform and the maximal shortening velocity (162, 409, 410, 600, 713). Ablation of the SM-B form in transgenic mice has been shown to result in a slower smooth muscle phenotype (48, 323, 323). Comparative studies and studies on transgenic animals cannot entirely exclude that alterations in other proteins contribute to the modulation of shortening velocity. The difference in velocity between the SM-B and SM-A forms in the in vitro motility assay is about twofold (560), which is less than the almost sevenfold difference between fast and slow smooth muscle preparations (437). This can suggest that other factors, in addition to the myosin heavy chain insert, influence velocity in the organized contractile system; such candidates include the essential myosin light chain and nonmuscle myosin heavy chain isoforms (see below), as well as thin filament-associated proteins, e.g., calponin (308, 456, 510, 510, 640). Urinary bladder tissue has a comparatively high expression of the inserted myosin isoform with ~80–90% SM-B at the mRNA level (30, 713), which would be consistent with the urinary bladder being a comparatively fast smooth muscle type. Newborn bladders have slightly lower content of SM-B, and culture of bladder myocytes decreases the content further (30).

Smooth muscle expresses two types of essential light chains, the acidic form LC_{17a} and the basic, nonmuscle LC_{17b}, which randomly can combine on the myosin heavy chain (100, 264, 275, 327, 374). These two isoforms are formed by alternative splicing, are of equal size, and differ in the carboxy-terminal nine amino acids (489). A large variation in the relative content of LC_{17a} and LC_{17b} exists between smooth muscles (275, 437, 635). A correlation between smooth muscle expression of essential light chain variants, and both the maximal shortening velocity and the ATPase activity have been found; high LC_{17b} content correlated with low shortening velocity and low ATPase activity (275, 437, 600). Extraction/reconstitution of essential light chain isoforms in muscle fibers and overexpression in isolated cells have shown a slowing effect of LC_{17b} on contractile kinetics (288, 457). Other comparative studies on isolated cells (586), in vitro motility experiments using expressed myosin heavy meromyosin (HMM) fragments, and essential light chain exchange experiments on isolated myosin have failed to show effects of the essential light chain composition on cell shortening velocity, ATPase, and actin translocation velocity (335, 553). In view of the negative in vitro motility data regarding effects of essential light-chain exchange and the strong evidence for effects of myosin heavy chain insert, the essential light chain isoform expression does not seem to be the primary modulator of contractile kinetics in smooth muscle. Comparative studies of smooth muscles show a correlation between both the essential light chain and inserted heavy chain composition (cf. Refs. 37,

612), and it is possible that the expression of these two forms is coregulated in the smooth muscle. A high LC_{17b} content and low SM-B content correlate with a slow and economical smooth muscle phenotype. How these structures interact in the organized system is not known. The relative LC_{17b} content is low in the urinary bladder tissue, ~10% in rat (600) and 20% in mouse (598), which is consistent with a comparatively fast smooth muscle phenotype.

In addition to the smooth muscle myosin heavy chain described above, smooth muscle can express type II filament-forming nonmuscle myosin isoforms (232, 366, 373). Two separate genes generate the two main nonmuscle myosin heavy chains, type A (NM-MHC-A, molecular mass 196 kDa) and type B (NM-MHC-B, molecular mass 198 kDa) (328, 331, 366, 595). The NM-MHC-A form is also expressed in other cell types, e.g., fibroblasts and platelets, and is upregulated in smooth muscle cell culture (329). The NM-MHC-B form, which is also denoted smooth muscle embryonic (SM_{emb}), is expressed in smooth muscle cells during development and in atherosclerotic plaques (366). The NM-MHC-B is expressed as an inserted larger isoform (molecular mass 229 kDa) in nervous tissue (641). Recently, the presence of a novel conventional myosin heavy chain with similarities to non- and smooth muscle heavy chain has been proposed on the basis of an analysis of the human genome (57); its tissue expression and cellular functions are currently not known.

The expression of nonmuscle myosins is low in adult urinary bladder [~10% of total heavy chain in the rat (440) and low also in other species (118, 408)]. Nonmuscle myosin is found in nonsmooth muscle cells in the serosa and urothelium and in a few cells in the interstitium between muscle bundles. In smooth muscle of newborn bladders, NM-MHC-A and low levels of NM-MHC-B are expressed, whereas in the adult smooth muscle cells only NM-MHC-A is found (30, 116, 408). The functions of the nonmuscle myosins in the urinary bladder are not known in detail. The NM-MHC-B is present in several organ systems during development, and ablation of the NM-MHC-B gene results in high prenatal lethality and severe cardiac and neurological disorders, suggesting that myosin form is important for development. The NM-MHC-A and NM-MHC-B are upregulated in urinary bladder smooth muscle cells in cell culture, suggesting that they can be important for smooth muscle cell migration and proliferation (30). In a recent study by Morano et al. (474), a mouse model was introduced where the smooth muscle myosin was ablated. The animals are born alive but die a few days after birth. Urinary bladder muscle from such animals can contract by the action of the nonmuscle myosins and provides a unique model for analysis of nonmuscle myosin function. The nonmuscle myosin forms filaments in these smooth muscle cells and supports a contraction with a slow onset and a low shortening velocity (408, 474). These results show that nonmuscle myosin also can have a contractile function in smooth muscle, which can be important during fetal life or in adult tissue with high content of nonmuscle myosins, e.g., large elastic arteries. In view of the low nonmuscle myosin content in the adult urinary bladder, it seems however unlikely that this myosin form contributes significantly to force or shortening of the adult urinary bladder. Nonmuscle myosin might also be a marker for nonsmooth muscle cells in the urinary bladder which have been suggested to differentiate into smooth muscle cells or have special functions during urinary bladder hypertrophy, as discussed below.

2. Cytoskeleton and intermediate filaments

The cytoskeleton in smooth muscle provides a structural framework for the cell as well as membrane attachments (cf. review in Ref. 605). The dense bodies are associated with a network of the cytoskeletal (10 nm) intermediate filaments (66, 665). In addition to the cellular contractile domain, composed of myosin and smooth muscle α-actin, a cytoskeletal domain composed of the intermediate filaments, nonmuscle β- and γ- actins, filamin, and calponin has been distinguished (418, 419, 508, 604). In the cell membrane, the cytoskeleton forms sites for contact with the cell environment. The dense bands in smooth muscle are cell adhesion complexes and are associated with several structural proteins, including, e.g., α-actinin, actin, filamin, calponin, vinculin, tensin, and integrins (cf. Ref. 605). These very complex and dynamic structures have mechanical roles in transmitting force from the contractile machinery to the surrounding cells and matrix, but also to receive and generate signaling information, e.g., for gene expression, cell migration, cell growth, and adaptation. The adhesion complexes, their links to the cytoskeleton, and role in signaling will not be covered further in this review but have been reviewed elsewhere (224, 303, 731, 744).

The intermediate filaments in smooth muscle are mainly composed of the proteins desmin and vimentin, although other intermediate filament proteins, e.g., cytokeratins, have been found. Vimentin is mainly found in smooth muscle of large arteries, and it is also present in mesenchymal nonmuscle cells, e.g., fibroblasts, whereas desmin is mainly found in intestinal smooth muscle and in the striated muscles (199, 212, 306, 378). Desmin and vimentin can coexist in the same smooth muscle cell (517, 663), and an interesting gradient in expression exists in the vascular tree, from mainly vimentin in the large arteries to more desmin in microarterial vessels (313, 518, 705).

The urinary bladder of rat and human contains predominantly desmin intermediate filaments (440, 441). Vimentin-positive nonmuscle mesenchymal cells are found

at the serosal and mucosal surfaces and in a limited number in the interstitium between the muscle bundles (86, 158, 599). The concentration of desmin in the mouse urinary bladder smooth muscle is more than 20-fold higher than in skeletal muscles (50). The ratio of desmin to actin contents is ~0.16 in the rat urinary bladder (440), which would correspond to a concentration ~6.7 mg/g smooth muscle cell.

The generation of desmin-deficient (Des −/−) mice (397, 466) has introduced new possibilities to study the function of the desmin intermediate filaments. These animals develop normally, showing that desmin is not required for normal muscle development. However, a cardiomyopathy with degeneration, calcification, and impaired cardiac function (51, 654) has been described. The structure of smooth muscle is essentially normal, except for the absence of intermediate filaments, showing that the desmin intermediate filaments are not required for development of a normal smooth muscle contractile phenotype (598). No compensatory upregulation of vimentin is observed. In the urinary bladder, passive tension in the muscle layer at optimal length for active force generation was slightly lower, suggesting that the intermediate filaments might have a small role in the support of passive bladder wall tension. Similar results have been reported for the passive wall tension in microarteries (705). However, passive tension could be well maintained over a large length interval showing that the intermediate filaments in the smooth muscle cells are not the only structures responsible for passive wall tension in the urinary bladder. The active force in the bladder wall was decreased to ~50% of normal, a change that could not be due to alterations in the content of contractile proteins or in the contractile activation systems. Similar results have been reported for cardiac muscle from desmin-deficient mice (51). These results suggest that the desmin intermediate filaments are responsible for transmission of active force in the smooth muscle cells, possibly by anchoring the contractile apparatus to the cell membrane or by coupling the dense body structures during active contraction.

C. Actin-Myosin Interaction

During force generation and shortening of muscle, the myosin cross-bridges interact with actin and hydrolyze MgATP to the products MgADP and P_i. The energy is supplied by the cell metabolism keeping the MgATP high while lowering the product concentrations, thereby shifting the MgATP hydrolysis reaction from its equilibrium. The energy is released in a multistep enzymatic process, where the myosin (M) cross-bridges bind and attach to actin (A) in a cyclic manner. The structural and biochemical events of this process in skeletal muscle have been reviewed in detail elsewhere (128). In smooth muscle the cross-bridge interaction is considered to follow the same general scheme of reactions as that proposed for skeletal muscle, although the rates of some reactions are different (cf. Refs. 39, 447).

In the absence of substrate, myosin binds strongly to actin, forming the rigor (A.M) complex in skeletal and smooth muscles. In living smooth muscle cells, the relative population in the rigor state is low, the A.M complex is rapidly dissociated by MgATP. This cross-bridge dissociation reaction is slightly slower in smooth compared with skeletal muscle, the second-order rate constant being $\sim 1 \times 10^5\ M^{-1} \cdot s^{-1}$ in the smooth muscle from rabbit urinary bladder (342), compared with $\sim 5 \times 10^5\ M^{-1} \cdot s^{-1}$ in the rabbit psoas muscle (235). The rate is slower in the slower, tonic, smooth muscle type of the femoral artery (342) compared with the faster phasic bladder smooth muscle. However, the MgATP-induced dissociation is rapid enough not to limit the rate of cross-bridge turnover or the shortening velocity in smooth muscle at normal MgATP concentrations (cf. Ref. 411), and it is not likely that this reaction is primarily responsible for the difference in contractile kinetics between skeletal and smooth muscles and between fast and slow smooth muscle types.

The cross-bridge power stroke is considered to be associated with release of P_i from the actin-myosin-ADP-P_i (A.M.ADP.P_i) state in skeletal and smooth muscle (281, 520). The active force of smooth muscle can thus be inhibited by high P_i concentrations and by the compound 2,3-butanedione monoxime (BDM), which interferes with the P_i release reaction (520, 521). Interestingly the effects of added P_i and BDM are lower in smooth compared with skeletal muscle and lower in slow compared with fast smooth muscles, possibly suggesting that the A.M.ADP state immediately after P_i release is less populated or that the binding constant for P_i is lower in muscles with slower myosins. This relationship seems to extend also to the nonmuscle myosins (408), where force is little affected by addition of P_i. The P_i release reaction is not rate-limiting for the maximal shortening velocity, since addition of P_i does not influence velocity (411, 520). Smooth muscle has been shown to generate higher force per myosin head than skeletal muscle (487). However, the cross-bridge power stroke of smooth muscle myosin generates ~10 nm unitary displacement or a unitary force of 1 pN (246, 377), values which are similar to those found in skeletal muscle myosin. Thus the difference in force-generating ability between smooth and skeletal muscles does not reside in the molecular mechanics. Instead, the higher force per myosin head in smooth muscle is due to the kinetics of the interaction (246). The relative time spent in attached force-generating states (duty cycle) is longer for smooth muscle cross-bridges. Also when fast and slow smooth muscle HMM, i.e., with and without the seven-amino acid insert in the myosin head region, are

compared with an optical trap method, similar unitary forces are found, but the duty cycle was longer in the noninserted myosin (377).

The maximal shortening velocity (V_{max}) of muscle reflects the maximal rate at which the myosin can propel the actin filaments under unloaded conditions. In general, shortening velocity of the comparatively fast urinary bladder smooth muscle [e.g., rat detrusor: 0.2 muscle lengths (ML)/s at 22°C (600)] is more than 10-fold slower than that of the fast skeletal muscle (129). A large span in shortening velocity also exists within the smooth muscle group, where the slow aorta muscle is about fivefold slower than the fast smooth muscles (cf. Ref. 437). This maximal shortening velocity is considered to be rate-limited by the cross-bridge dissociation after the power stroke, and a strong correlation exists between the rate of the ADP release reaction and the maximal shortening velocity (593, 707). In smooth muscle preparations with actively cycling phosphorylated myosin, MgADP inhibits shortening velocity (411), showing that kinetics of the MgADP reaction can limit shortening velocity of smooth muscle. In vitro the MgADP binding to smooth muscle myosin in the presence of actin is strong compared with that of skeletal muscle myosin (139). In optical trap measurements and in in vitro motility assays, the rate of ADP release is approximately two- to fivefold slower in smooth muscle myosin without the heavy chain insert compared with the inserted myosin (377). In smooth muscle fiber preparations a strong binding of ADP to rigor cross-bridges has been reported (32, 502), with a binding constant in the micromolar range. The comparatively fast (phasic) urinary bladder smooth muscle has a lower binding of ADP in rigor (206) and in the dephosphorylated state during relaxation compared with the slower (tonic) arterial muscle (340). A markedly stronger MgADP binding is also observed during active cross-bridge cycling in slow compared with fast smooth muscles (411). Thus, in general, MgADP binding is stronger in slow compared with fast smooth muscles and in smooth compared with skeletal muscle. The kinetics of the MgADP reaction can be responsible for the modulation of shortening velocity of the organized contractile system in smooth muscle. Interestingly, isolated smooth muscle myosin exhibits unique structural changes associated with MgADP binding (236, 714). These properties have implications for analysis of the conversion energy in the myosin cross-bridge (cf. Ref. 222), although analysis of the MgADP binding reaction in vitro suggests that the MgADP release is not likely to be coupled to the force generation (139). The mechanical effects of MgADP binding to smooth muscle preparations in rigor are controversial. No mechanical effects were noted by Dantzig et al. (144), whereas a decrease in rigor force upon MgADP binding was observed by Khromov et al. (341) in both arterial and urinary bladder smooth muscle.

The kinetics of the urinary bladder contractile system thus reflect a comparatively fast smooth muscle phenotype. The maximal shortening velocity of adult urinary bladder is ~80% of the fastest mammalian smooth muscle found (rabbit rectococcygeus, Refs. 408, 437, 600). In relation to the slower smooth muscle phenotypes, e.g., in arteries, the urinary bladder muscle has a comparatively low affinity for MgADP (206, 340), high affinity for MgATP (342), and high phosphate sensitivity of active force (408, 411). These properties of the reaction(s) determining the force generation and the shortening velocity are correlated with the myosin expression (cf. sect. III*B1*), with low essential light chain LC_{17b} content and high content of inserted myosin heavy chain (SM-B).

D. Energetics and Cell Metabolism

ATP is the immediate substrate for the different processes involved in contraction and relaxation of the urinary bladder muscle, from membrane pump activity, Ca^{2+} handling, phosphorylation processes, to cross-bridge cycling. The cellular ATP concentration is maintained by mitochondrial respiration, glycolysis, and conversion of the high-energy compound phosphocreatine (PCr). Reviews regarding high-energy phosphates and cellular energy metabolism in smooth muscle have been presented (91, 531, 675, 676). The cellular concentrations of ATP and PCr in smooth muscle are generally low compared with skeletal muscle. In particular, the PCr is lower in smooth compared with skeletal muscle (531). The contents in urinary bladder tissue determined by biochemical assays are within the range observed for different smooth muscle tissues [relaxed rabbit urinary bladder (in μmol/g): ATP, 0.79; ADP, 0.15; P_i, 1.3; PCr, 1.76; Ref. 274], although some species-dependent differences might exist (386). Data from urinary bladders have also been obtained using ^{31}P-nuclear magnetic resonance (NMR) measurements (274, 368). Hellstrand and Vogel (274) reported that NMR measurements gave similar PCr/ATP ratios, but lower values for ADP and P_i, compared with biochemical measurements, which might reflect an intracellular compartmentalization of the latter compounds.

The cellular concentration of ATP is well maintained during sustained contractions of urinary bladder muscle although PCr is decreased by 10–30% (274). In uterine tissue it has been reported that contractions are associated with decresaed ATP and PCr and incresaed P_i levels (371). A pronounced decrease in ATP and PCr and increase ADP was reported after metabolic inhibition of the urinary bladder tissue (274). Lowered cellular MgATP concentration would influence several processes in the bladder muscle cell. The actin myosin interaction in the cross-bridge cycle can operate at very low [MgATP], and the primary effect of lowered [MgATP] in severe ischemia

or during impaired metabolic activity would rather be a reduced myosin light-chain phosphorylation and decreased activation of the contractile system (273). Increased concentrations of phosphate could theoretically inhibit force; however, it is unlikely that the cellular concentration of phosphate can reach high enough levels to have an effect on force in fast smooth muscle (cf. Ref. 520). The [MgADP] in smooth muscle increases during prolonged contraction and in ischemia (192, 365). The binding of MgADP to the smooth muscle actin-myosin complex is very strong, and shortening velocity of smooth muscle is inhibited at low MgADP concentrations as discussed above. A physiological effect would be that shortening velocity and possibly also relaxation, rather than active force, are inhibited at the cross-bridge level by small increases in [MgADP]. This effect would be stronger in hypertrophic urinary bladder smooth muscle where the detrusor muscle changes toward a slower contractile phenotype, in which MgADP binding is stronger (see sect. III*B1*). Whether the cellular ADP can inhibit shortening velocity at unchanged force also during sustained contractions under conditions with normal energy supply is not clear.

The metabolic substrates utilized by the urinary bladder smooth muscle in vivo are not known. For vascular smooth muscles, measurements of the respiratory quotient suggest that glucose is an important source of energy, although several other substrates may also be metabolized (531). The uptake of glucose in smooth muscle is considered to involve the GLUT1 glucose transporter, which is different from the GLUT4 type present in the insulin-sensitive striated muscle and adipose tissue (cf. Ref. 444). However, some insulin sensitivity has also been observed for the GLUT1 type. A small effect of insulin on the glucose uptake in bladder tissue has been described (269). An interesting aspect of smooth muscle glucose metabolism is that a large fraction of the glucose is metabolized to lactate also under aerobic conditions, possibly to supply membrane pump activities (cf. Refs. 98, 417). In the rabbit urinary bladder in vitro, 81% of the glucose is metabolized to lactate, whereas ~11% is oxidized to CO_2 and 4.7% converted to glycogen (269). Since the ATP yield is severalfold higher for aerobic metabolism of glucose, the oxidative metabolism is the main source of ATP under normal conditions. In the relaxed rat urinary bladder in vitro in the presence of glucose, the oxygen consumption is ~1.5 nmol $\cdot$ min^{-1} $\cdot$ mm^{-3}, and lactate production is 0.5 nmol $\cdot$ min^{-1} $\cdot$ mm^{-3}. During active contractions these rates increased about two- and threefold, respectively (41). With the use of these values, it can be calculated that ~5–10% of the ATP in the urinary bladder is derived from the aerobic glycolysis to lactate (41).

The formation of lactate in muscle is catalyzed by lactate dehydrogenase (LDH). This enzyme exists as a tetramer with different combinations of the two M and H polypeptide chains (184), thus creating five different LDH isoforms. LDH with high M content is more directed toward formation of lactate and is found in fast skeletal muscle, whereas LDH with more of the H form is product inhibited by lactate and associated with slower aerobic striated muscles, e.g., the soleus and the heart (184, 283, 542). The rat urinary bladder smooth muscle has an LDH isoform pattern with less of the H form compared with the slow aorta smooth muscle (442), which suggests, in analogy with the situation in striated muscle, that the expression of enzymes in the cellular metabolism are correlated with the contractile properties. However, this is not a general correlation for all smooth muscles, since the different layers of the rabbit urethra, which have markedly different shortening velocities, exhibit similar LDH isoform patterns (42). Also under pathophysiological conditions the shortening velocity and LDH isoform expression pattern change in opposite directions, with more of the M-form being associated with lower shortening velocity (442, 600).

The intracellular pH has been measured to be ~7 in isolated human detrusor muscle cells (201). During hypoxia in the detrusor active force is lowered (e.g., Ref. 41). In a study by Thomas and Fry (653a) it was shown that the lower detrusor force in hypoxia was associated with a transient initial intracellular alkalosis and a decreased extracellular pH. Extracellular acidosis is associated with decreased force generation of the detrusor in contrast to intracellular acidosis, which increases force (202, 406). The force generation, shortening properties, and myofilament Ca^{2+} sensitivity of the contractile system is little affected by variations in pH (33, 725). The lower force in extracellular acidosis has been attributed to attenuating effects on Ca^{2+} influx through L-type channels, which also affects release from the intracellular stores (202). Intracellular acidification has been shown to increase force via improved Ca^{2+} uptake and and release from intracellular stores (724).

E. Detrusor Muscle Mechanics

The bladder wall undergoes large changes in extension during normal filling and emptying. Isolated urinary strips of the urinary bladder wall can be examined in vitro to determine the relation between length and wall tension (674). These data for the relation between length and force can be converted to volume and pressure data using the law of Laplace and assuming a model for the bladder shape and for how the wall stretch is distributed in the bladder wall. An assumption of a spherical bladder shape, with an incompressible wall and isotropic homogeneous stretch, can give a good description of the bladder mechanics during filling (cf. Ref. 140).

The bladder muscle wall exhibits a nonlinear relation between stretch and passive tension. At lengths above that where maximal active force is recorded, the passive tension increases steeply (598, 671). Some differences between species in the properties of the length-tension relations have been reported (413). The length-tension relations determined in vitro are usually performed using slow stretch or longer equilibration periods at each length, which gives data reflecting the passive behavior of the bladder during slow filling in vivo. During fast stretch or extensive deformation, the bladder wall also exhibits viscous and plastic behavior (9, 131, 191, 360). These viscous properties would be involved in "stress relaxation" phenomena observed in the whole urinary bladder or isolated muscle strips. A rapid increase in length, or volume, results in a fast rise in force and pressure and a subsequent slow return to the original levels. A rapid decrease in length or volume would give an immediate drop in force and pressure followed by a gradual increase.

The cytoskeleton of the smooth muscle cells might contribute with a small component of the passive tension in the range of wall stretch where active tension is near maximal (598). It should also be noted that a fully relaxed state with a complete absence of cross-bridge interaction might not be attained in the living smooth muscle cells, and thus passive or relaxed properties of the bladder wall might contain a small contribution of cross-bridge interaction. However, the passive viscoelastic properties are considered to be mainly due to properties of the extracellular matrix in the bladder wall. Main extracellular components in the urinary bladder are elastic fibers and collagen fibrils, which are present in the serosa, between the muscle bundles, and in small amounts between the smooth muscle cells in the muscle bundles (214). The collagen fibrils in the urinary bladder are formed by collagen type I and III (185, 343) and the elastin fibers of elastin from the soluble precursor tropoelastin (383).

The active force of the bladder muscle is dependent on the wall stretch. The relation between muscle length and active force is comparatively broad, and bladder muscle from experimental animals and humans can generate force over a large length interval (413, 443, 672). The different extents of wall stretch are coupled with corresponding changes in cell length, which indicates that slippage of the cells in the bladder wall does not occur during the length changes (677); however, at short lengths, the cells might not be aligned along the long axis of the preparation (674). The length dependence of the active force most likely reflects that filament overlap and cross-bridge interaction is dependent on muscle length. In addition, the muscle stretch can influence the excitation-contraction coupling resulting in a less optimal activation at short lengths (36, 251). To obtain a measure for the maximal active force generation of the smooth muscle component, the preparations have to be examined at optimal length and corrections performed for the content of smooth muscle in the preparations. At optimal length the detrusor muscle has been reported to generate ~200 mN/mm^2 smooth muscle area in human detrusor (443), 590 mN/mm^2 smooth muscle area or 5.5 μN/cell in guinea pig detrusor (677), 80 mN/mm^2 in rat detrusor (674), and 60 mN/mm^2 in mouse detrusor (598). For comparisons of cellular force generation of detrusor muscle from control and pathophysiologically altered bladders, several parameters, including the extent of stretch, the content of smooth muscle cells, and the degree of activation, have to be considered. The cellular force generation is the result of the number of cross-bridges acting in parallel and the intrinsic force generation of the cross-bridge. As discussed in section III*C*, a longer duty cycle seems to be one mechanism responsible for the comparatively high force output per amount of myosin in smooth muscle.

As discussed in section III*C*, the V_{max} gives information regarding the rate of filament sliding. If the muscle is maximally activated, the V_{max} is considered to reflect the kinetics of the myosin cross-bridge interaction. Thus V_{max} varies between smooth muscles and can change during physiological adaptation. However, the maximal shortening velocity is also modulated by several factors in the living tissue. The velocity is dependent on the mode of activation, the time after stimulation, and muscle length (cf. Ref. 43), showing that an individual smooth muscle can modulate the cross-bridge turnover. Experiments on permeabilized smooth muscle have shown that both $[Ca^{2+}]$ and myosin light-chain phosphorylation can alter both V_{max} and force (34, 438). One important concept in this context is the "latch" phenomenon (149), where cross-bridges are suggested to attach in nonphosphorylated state and thereby lower velocity and maintain tension at low ATP turnover. Several mechanisms have been proposed to be involved in the latch state, a dephosphorylation of attached cross-bridges, additional regulatory systems, or possibly metabolic factors (cf. Ref. 43).

The velocity of filament sliding in striated skeletal muscle has been shown to be independent of the filament overlap (166). Experiments of fully activated human permeabilized urinary bladder strips (443) and electrically activated intact pig urinary bladders (469) suggest that the maximal shortening velocity is not length dependent. These results would fit with the view that cross-bridge kinetics under unloaded conditions are not dependent on the number of cross-bridges acting in parallel. In an early study Uvelius (672) reported, however, that the V_{max} of high-K^+ activated intact rabbit urinary bladder is dependent on the length. This finding suggests that in the intact bladder wall velocity can be influenced by passive components or length dependence of the activation systems.

The V_{max} of intact urinary bladder smooth muscle preparations has been estimated to be in the range 0.3–0.4 ML/s at 37°C in different species (rabbit, Refs. 672, 678;

guinea pig, Ref. 243). It was early recognized in urinary bladder muscle that the mode of activation influences shortening velocity (678). The velocity was higher after electrical stimulation compared with high-K^+ activation. This is most likely a reflection of the activation-dependent modulation of V_{max} discussed above. To determine the V_{max} of the fully activated contractile machinery, experiments have been performed on permeabilized urinary bladder smooth preparations where the environment of the contractile proteins can be held constant and a maximal myosin light-chain phosphorylation can be achieved. Under these conditions at 22°C, V_{max} is reported to be ~0.2 ML/s in the human urinary bladder (443), in the rat (600, 602), and in the mouse (598).

The relation between active force, or the load on the muscle, and the shortening velocity is described by a hyperbolic relationship (282), where V_{max} is the velocity at zero load and the isometric force (P_o) is the force at zero velocity. Since both P_o and V_{max} are regulated, different force velocity curves, with different V_{max} and P_o, can be obtained depending on the contractile activation (cf. Ref. 685). In the contracting urinary bladder wall during emptying, the detrusor muscle wall will thus operate along these force-velocity relationships depending on luminal pressure, bladder volume, and state of activation.

F. Pathophysiological Adaptations

Hypertrophy and hyperplasia of the smooth muscle in the urinary bladder can occur in response to urinary outlet obstruction, e.g., in benign prostatic hypertrophy, or after decentralization, e.g., in spinal cord injuries. The primary factor for induction of the growth seems to be stretch of the bladder wall components, although the cellular signals mediating the growth response are not fully characterized. Several receptors, signaling pathways, and growth factors might be involved, e.g., insulin-like growth factor I (IGF-I) or its binding proteins (1, 2, 109–111), epidermal growth factor (EGF) (690), heparin-binding EGF-like growth factor (HB-EGF) (501), angiotensin II receptors (527), basic fibroblast growth factor (bFGF) (107, 108), or altered Ca^{2+} handling (367).

Hypertrophic growth of detrusor muscle in response to urinary outlet obstruction is well documented in humans (229) and has been extensively studied in several animal models where a partial obstruction is applied to the urethra (80, 389, 460, 483, 591, 621, 718). Urinary bladder distension and growth is also found in animals with other modes of distension or increased urine volume load, e.g., rats with hereditary diabetes insipidus (435), rats with pharmacologically induced diabetes mellitus (40, 111, 402, 673), osmotic diuresis (412), application of a paraffin bolus in the bladder (539), and preganglionic denervation or removal of pelvic ganglia (58, 171, 172). Some of these conditions can influence the bladder structure via metabolic pathways. However, because denervated urinary bladders can hypertrophy and because this growth can be prevented by manually emptying the bladder (58, 431), the results clearly show that trophic influences of parasympathetic nerves or active contractions by the bladder muscle are not required for hypertrophic growth and are consistent with wall stress being the primary initiator of growth.

The growth of the urinary bladder in response outflow obstruction can be quite dramatic, e.g., in the rat the urinary bladder weight increases from the normal 80 mg to ~140 mg in 3 days, 170 mg in 10 days, 640 mg in 42 days, and 1,000 mg in 90 days (550). The increase in bladder mass in response to outflow obstruction is reversible; when the obstruction is removed in experimental animals, the bladder regains almost normal weight and protein composition is normalized (405, 441, 702), although the deobstructed urinary bladders show differences in cellular and intercellular structure compared with both control and hypertrophic urinary bladders (215).

In a study by Gabella and Uvelius (214), the fine structure of normal and hypertrophic rat urinary bladder was extensively characterized. It was shown that the muscle bundles became larger and longer and that the transverse area of the smooth muscle cells increased, suggesting hypertrophy of the cells. No mitoses were found, although cells with two nuclei were present. Gap junctions were very few or absent both in control and hypertrophied tissues.

The bladder hypertrophy can also be associated with alterations in extracellular materials. In the trabeculated bladder from patients with prostatic enlargement, Gosling and Dixon (238) found an increase in extracellular material and collagen. In the hypertrophic rat urinary bladder, total collagen increases although the concentration of collagen appears to decrease (679).

Hypertrophy of smooth muscle cells seems to be a major cause for the growth of the urinary bladder wall in response to urinary outflow obstruction (214), although measurements of DNA content suggest some contribution of smooth muscle hyperplasia (680). Proliferation of mesenchymal nonmuscle myosin heavy chain A (NM-MHC-A, see above), vimentin, and smooth muscle α-actin positive cells of the serosa has been described (86, 118). These cells have been suggested to mature towards adult smooth muscle cells in hypertrophic growth and after bladder wall injury (186, 557). These results suggest that bladder muscle has the ability to generate new smooth muscle cells that can be important in some pathophysiological conditions, e.g., in regeneration bladder wall after injury (186), after insertion of acellular grafts (547, 548), or after partial cystectomy (200), although such smooth muscle hyperplasia is most likely not the major cause for

the growth of the detrusor muscle in urinary outflow obstruction.

The hypertrophic growth of the smooth muscle cells in the urinary bladder in response to outflow obstruction is associated with increased total concentrations of the contractile proteins (58, 440). In the hypertrophying rat urinary bladder, the synthesis of myosin appears, however, at some stages of growth not to be in pace with the increase on smooth muscle volume, resulting in a lower concentration of the contractile protein, which is correlated with a decreased active force per muscle area in hypertrophic tissue (41, 440). The myosin isoform pattern is changed during hypertrophic growth of the urinary bladder. A decrease in the SM2/SM1 ratio has been reported for obstructed urinary bladder of rabbit and rat (88, 118, 440, 441, 571, 702), whereas in biopsy samples from patients with bladder hypertrophy an increase has been reported (441). The actin isoform expression pattern has been reported to change towards a distribution with less smooth muscle α-actin and more γ-actin in hypertrophic rat bladder (440) and towards less β-actin more γ-actin at unchanged α-actin in hypertrophic rabbit bladder (345). In obstructed urinary bladder of patients a significant increase in α-actin and a decrease in β-actin has been found compared with control bladders (441). The functional consequences of a change in actin isoform distribution and myosin SM2/SM1 ratio in smooth muscle are not clear at present. However, these isoforms do not seem to be major determinants of contractile kinetics as discussed in section III*B1*. The 17-kDa essential light-chain expression changes towards more of the LC_{17b} form and the amount of the inserted myosin heavy chain (SM-B) decreases in hypertrophic urinary bladder (600), changes which would be associated with a slower, more economical contractile phenotype. Early studies of actin-activated myosin ATPase in vitro (571) or tension-associated ATP turnover in bladder muscle preparations (41) suggested that actin-myosin cross-bridge turnover was not altered. However, recent determinations of the force-velocity relationship show that the maximal shortening velocity is decreased in hypertrophic urinary bladder (600, 626), which suggests that the changes in myosin isoform expression pattern result in a slower, more economical muscle.

A striking feature in several forms of hypertrophy in visceral smooth muscle is an increase in intermediate filaments (213). This is also evident in the hypertrophying urinary bladder of experimental animals and in humans, where the amount of the intermediate filament protein desmin increases relative to other contractile and cytoskeletal proteins (58, 440, 441, 690). The intermediate filament protein vimentin is much less abundant in the urinary bladder and is mainly found in cells at the mucosal and serosal surfaces and in the interstitium (e.g., Ref. 599). Vimentin in the urinary bladder has been reported to increase also in hypertrophy (118, 440). The mechanical function of the increased number of intermediate filaments and desmin in hypertrophying urinary bladder is unknown. The presence of, and increase in, intermediate filaments are not required for the hypertrophic growth, but possibly these structures are important for maintenance of cellular structure, as cell size increase (R. Sjuve and A. Arner, unpublished data).

The hypertrophy of the urinary bladder wall involves a thickening of both epithelium, muscle layer, and serosa. The tissue is well vascularized (214), suggesting formation of new blood vessels in the vascular wall. Microarterial vessels supplying the bladder also grow in size (64). Blood flow has been reported to increase to the rabbit bladder initially during hypertrophy (398). However, after 2 wk of obstruction, microcirculation has been found to be impaired (659) and in chronic decompensated hypertrophic urinary bladders blood flow is decreased (576). The content of mitochondrial enzymes and oxidative metabolism have been reported to decrease in hypertrophic rabbit urinary bladder and in urinary bladder from men with benign prostatic hyperplasia (60, 268, 388, 399). Decreased aerobic metabolism (326) and lowered cellular ATP and PCr levels have been reported (387). It has been suggested that altered mitochondrial function is involved in the bladder pathology associated with benign prostatic enlargement (499) and that structural and functional changes can reflect hypoxia in the wall of the urinary bladder (241). In contrast, in the rat urinary bladder the number of mitochondria in the hypertrophic cells increase, keeping the relative mitochondrial volume and the amount of mitochondrial enzymes per unit weight unchanged (141, 214). These results thus show that hypertrophy of the detrusor muscle can occur without major mitochondrial dysfunction. It is likely that the responses of the bladder wall are very complex and that the extent of structural changes and wall hypoxia/ischemia varies between species and with time and severity of the obstruction. In the hypertrophied rat urinary bladder active force is better maintained under hypoxic conditions (41). The LDH enzyme pattern changes towards more of the M-form, which is more directed toward formation of lactate (442) and the contractile system changes towards a more economical phenotype (see sect. III*D*). These changes possibly reflect adaptations to impaired energy supply in the hypertrophying urinary bladder wall. A summary of the changes in contractile properties induced in the detrusor by adaptive growth is given in Figure 2. Changes in receptors and activation systems are presented in the subsequent sections.

Urinary bladder function is altered in several other (patho)physiological conditions. In pregnancy urinary incontinence (particularly stress incontinence) is common and has been attributed, at least in part, to changes in bladder and urethral function (660). In rats, pregnancy

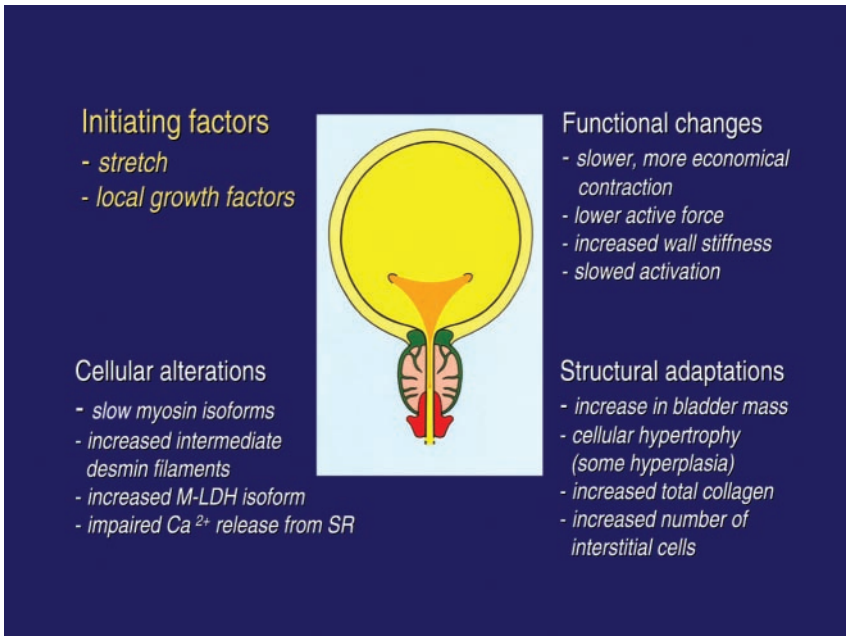

FIG. 2. Changes induced in the detrusor by adaptive growth. SR, sarcoplasmic reticulum; LDH, lactate dehydrogenase.

was reported to increase bladder weight and capacity, decrease the responses to α-adrenoceptor stimulation, and increase the response to ATP (658). A lowered bethanechol-induced active force has been reported (394, 745). These changes are associated with a lowered muscarinic receptor density on urinary bladders (53, 394). To what extent the receptor and functional changes demonstrated in the pregnant bladder of different species can explain the voiding disturbances found in pregnant women remains to be established.

IV. EXCITATION-CONTRACTION COUPLING

A. Regulation of Contractile Proteins

Smooth muscle contraction is initiated by an increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) (cf. Ref. 563). In principle, Ca^{2+} can enter the cytoplasm through the cell membrane, via Ca^{2+} channels, or be released from the sarcoplasmic reticulum (SR). These pathways for Ca^{2+} translocation have been the topic of several recent excellent reviews (65, 73, 217, 362, 572, 615).

The release of Ca^{2+} from the SR is an important step in activation of the detrusor muscle. This is evident from studies using blockers of SR function showing that both nerve- and agonist-induced contractions are dependent on a functional SR (142, 395, 485). The release of Ca^{2+} is triggered by inositol trisphosphate (IP_3) via IP_3 receptors and by Ca^{2+} (Ca^{2+}-induced Ca^{2+} release) via ryanodine receptors (cf. Ref. 73). Interestingly, stretch of rabbit urinary bladder smooth muscle cells has been shown to activate Ca^{2+} release via gating of the ryanodine receptor, which suggests that stretch-induced Ca^{2+} release can be a further mechanism influencing Ca^{2+} release (312). The activity of the SR Ca^{2+}-ATPase in the [SERCA] is inhibited by the associated protein phospholamban, and depletion of this protein leads to altered bladder contractility (504), suggesting that the content of phospholamban can be a factor modulating bladder contractility. In addition to releasing activator Ca^{2+}, the SR can influence bladder contractility via modulating the K^+ channel activity and thereby promote relaxation (cf. sect. v*C2*) and by introducing a Ca^{2+} sink buffering the Ca^{2+} influx (741). The mechanisms of Ca^{2+} influx through Ca^{2+} channels are described in section v*B*.

The Ca^{2+} activation of the contractile proteins is considered to occur via a phosphorylation pathway where Ca^{2+} binds to calmodulin, and the Ca^{2+}/calmodulin complex activates the myosin light-chain kinase (MLCK) which catalyzes the phosphorylation of the 20-kDa myosin regulatory light chains on serine at position 19 (cf. Refs. 43, 216, 286, 625). Dephosphorylation of the regulatory light chain is performed by a myosin light-chain phosphatase (MLCP). The main pathways of cellular contractile activation are also shown in Figure 5 in context of the muscarinic receptor signaling. Several subtypes of the protein phosphatase exist (cf. Ref. 126). The phosphatase responsible for light-chain dephosphorylation in smooth muscle seems to be of type protein phosphatase (PP)1 or PP2A. PP1 has been isolated from smooth muscles including pig urinary bladder (588). The urinary bladder phosphatase (SMPP-1M, Ref. 588) is a trimeric protein composed of a 37-kDa catalytic PP1 subunit (PP1C) and two additional subunits of molecular mass 110–130 and 20

kDa, which is similar to the composition of the avian isoform (8, 262, 587). The larger 110- to 130-kDa subunit, which exists in different isoforms, is considered to have a regulatory function and is generally referred to as the myosin phosphatase targeting, binding, or regulatory subunit (MYPT).

The molecular mechanisms involved in the regulation of smooth muscle myosin by phosphorylation are not known, although it has been shown that myosin light-chain phosphorylation influences a step associated with the phosphate release (583). Cross-bridge cycling will occur while the myosin is phosphorylated. In a smooth muscle where the Ca^{2+}-induced activation of MLCK and the MLCP activity are the main pathways for contraction and relaxation, a relation between the $[Ca^{2+}]_i$, the extent of myosin light-chain phosphorylation, and force would exist. Several studies on smooth muscle preparations have shown that these three parameters change during contraction and relaxation (cf. Ref. 43). However, it has also become evident that the relation between Ca^{2+} concentration and myosin light-chain phosphorylation is very variable in intact muscle and can be directly altered by agonists in permeabilized muscle (354). This modulation of Ca^{2+} sensitivity involves several regulatory system influencing the MLCK and MLCP activities in the smooth muscle cell (cf. Refs. 616, 617). A decrease in MLCP activity, by Ca^{2+}-independent mechanisms, or an increase in MLCP activity would lead to a decreased Ca^{2+} sensitivity, and increase in MLCP activity by Ca^{2+}-independent mechanisms, or a decrease in MLCP activity would lead to a increased Ca^{2+} sensitivity. Several of the physiological agonists do not only change $[Ca^{2+}]_i$ but also change the sensitivity of the contractile system to Ca^{2+} via an extensive cellular signaling network (cf. Fig. 5).

The MLCK can be phosphorylated at a regulatory site A, which decreases the affinity for Ca^{2+}/calmodulin and decreases the Ca^{2+} sensitivity (624). The cAMP-dependent kinase (PKA) can phosphorylate MLCK in vitro (6, 624), although this mechanism does not appear to be responsible for the cAMP-induced relaxation of smooth muscle (624). The calcium/calmodulin-dependent protein kinase II (CaM kinase II) phosphorylates MLCK at site A, and this mechanism is operating in smooth muscles activated with different agonists (651, 686), resulting in a dual role for Ca^{2+}/calmodulin, an initial rapid activation of the MLCK, and a subsequent slower inactivation occurring at higher Ca^{2+}/calmodulin concentration. MLCK can also be phosphorylated by mitogen-activated protein (MAP) kinases (479) and p21-activated kinase (PAK), a member of the *rho* family of small GTPase-dependent kinases (233, 573). Whereas MAP kinase phosphorylation activates MLCK, PAK2 phosphorylation inhibits the enzyme. The effects of MAP kinase in isolated smooth muscle preparations are partly conflicting. An increased Ca^{2+}-activated force was reported by Gerthoffer et al. (226) using ERK2 MAP kinase, whereas Nixon et al. (503) found no effect using recombinant p42 MAP kinase. The role of these pathways in the living smooth muscle cells is not fully understood.

During recent years, regulation of the MLCP has received much attention. One main pathway for inhibition of the MLCP and inducing Ca^{2+} sensitization involves a specific kinase (*Rho*-associated kinase), which is activated via small G proteins of the *Rho* superfamily (cf. Ref. 614). The *Rho* (Ras homology gene, cf. Ref. 421) exists in three forms: *RhoA*, *RhoB*, and *RhoC* (634). It belongs to the *Ras* family of small GTPases and is similar to its relatives *Rac* and *Cdc42* (634). In principle, *Rho*, like other small G proteins, is activated by an exchange of the bound GDP for GTP. This exchange and activation/deactivation is controlled by several other proteins, including GDI (GDP-dissociation inhibitory factors), GAP (GTPase activating proteins), and GEF (guanine nucleotide exchange factors) (634). In the relaxed smooth muscle cell, *Rho* is thought to be present in cytosol as *Rho*-GDP-GDI complexes, and activation in the presence of GTP is catalyzed by *Rho*-GEF and leads to a translocation of *Rho* to the cell membrane (237, 614). Activation of *Rho* and Ca^{2+} sensititization can be achieved in the isolated protein and in permeabilized preparations using guanosine 5′-*O*-(3-thiotriphosphate) (GTPγS) (353). The agonist-induced Ca^{2+} sensitization of intact smooth muscle can be inhibited using cell-permeable toxins that ADP-ribosylate or glucosylate *Rho* (207, 415), further showing the importance of *Rho* in the Ca^{2+} sensitization of smooth muscle. *Rho* activates enzyme *Rho*-associated kinase (346, 455). Activation of *Rho*-associated kinase leads to phosphorylation of the myosin binding subunit of the MLCP and inhibits this enzyme (346). Recently a ZIP-like kinase, the MYPT kinase associated with the MLCP, has been described (420). This enzyme phosphorylates MYPT and has been proposed to be a link between the *Rho*-kinase and MYPT phosphorylation (69). The *Rho*-associated kinase, also denoted ROK, is identical to the *Rho*-associated coil kinase (ROCK) found in the brain and exists in two isoforms ROCK I (ROKβ) and ROCK I I (ROKα) (cf. Ref. 10). The pathway is most likely complex, *Rho* can have other targets, e.g., PKN (12), and *Rho*-kinase can phosphorylate other targets in the cell, e.g., the myosin regulatory light chains (11). Furthermore, the cellular localization and translocation is important for the action of the components in the signaling cascade (cf. Ref. 217). The Ca^{2+} sensitization of intact smooth muscle can be inhibited by the cell-permeant *Rho*-kinase inhibitors Y-27632 and HA-1077 (205, 632, 669, 740), showing that the *Rho*-kinase is an important step in agonist-induced activation of smooth muscle.

It has recently been shown that urinary bladder contains the two isoforms of *Rho*-kinase (ROCK I and ROCK II) (715) and that Y-27632 and HA-1077 can inhibit con-

tractions of rabbit urinary bladder smooth muscle induced by electrical stimulation, bethanechol, carbachol, and neurokinin A (311, 715). This suggests that the *Rho-Rho*-kinase pathway is involved in excitation-contraction coupling of muscarinic and tachykinin receptors in the urinary bladder. Interestingly, Y-27632 also inhibited spontaneous tone and responses to the P2X receptor agonist α,β-methylene-ATP of the rabbit urinary bladder (715), which might reflect effects of Y-27632 on other processes in the cell or that the spontaneous tone and P2X receptor responses in the urinary bladder include a component of activated or tonic *Rho*-kinase activity. The Rho-kinase pathway may be altered in pathophysiological conditions of the urinary bladder. Bing et al. (61) have reported that in decompensated bladders in outflow obstruction ROKβ is upregulated, which was suggested to contribute to slow relaxation and altered force maintenance.

The protein kinase C (PKC) can modulate the Ca^{2+} sensitivity of contraction via a *Rho-Rho*-kinase independent pathway (309). The CPI-17 is mainly expressed in smooth muscle tissues and, when phosphorylated, it is a potent inhibitor of the MLCP (180, 182, 585). CPI-17 is strongly phosphorylated by PKC and is suggested to mediate the Ca^{2+}-sensitizing effect of PKC and to be involved in the excitation following activation with histamine and phenylephrine (181, 352, 355). In addition to PKC, other kinases including PKN (255) and Rho-kinase (363) have been reported to phosphorylate the CPI-17. Both MYPT1 and CPI-17 are expressed in urinary bladder smooth muscle (722), although the CPI-17 content is lower than in arterial muscle. This correlates with lower Ca^{2+}-sensitizing effects of the PKC activator phorbol 12,13-dibutyrate (PDBu) in urinary bladder muscle (722).

The Ca^{2+}-sensitizing systems are targets for relaxant pathways. The cGMP and the cGMP-dependent kinase (PKG) can inhibit Ca^{2+}-induced contraction and Ca^{2+} sensitization and thus lead to relaxation (544, 545, 728). The targets for this mechanism are not fully resolved. The protein telokin is phosphorylated by PKG and PKA and has been proposed to be involved in relaxant action of cGMP and cAMP (727). Both PKA and PKG phosphorylate *Rho* (574, 575) and inhibit its Ca^{2+}-sensitizing action.

The mechanisms acting on MLCK and MLCP activities modulate active force by altering the Ca^{2+} sensitivity of contraction, i.e., the relation between intracellular free Ca^{2+} and light chain phosphorylation. These mechanisms would theoretically maintain a specific relationship between light chain phosphorylation and force. However, it was early recognized that also this relation could be altered (cf. review in Ref. 43). One such situation is sustained contractions in large arteries, where the initial force development was associated with increased light chain phosphorylation, whereas the sustained force was maintained at low phosphorylation levels. This finding where force thus can be maintained by low levels of phosphorylation was denoted "latch" (149). The latch state was also associated with a slow cross-bridge turnover and an economical tension maintenance. As proposed by Murphy and colleagues (149, 488), the dephosphorylated cross-bridges (the "latch" bridges) are formed by dephosphorylation of attached cross-bridges. These cross-bridges detach and cycle very slowly and can thus maintain force at low energy expenditure. However, other mechanisms might contribute to the slow cycling and high tension maintenance in prolonged contractions and in some tissues, e.g., cooperative attachment of cross-bridges (619), strong ADP binding (339, 411), contribution of nonmuscle myosin (474), or other Ca^{2+} regulatory systems not operating via myosin light-chain phosphorylation as discussed below.

Smooth muscle thin filaments contain actin and tropomyosin. Tropomyosin might be important for cooperative binding of myosin heads to actin, and tropomyosin in smooth muscle has been shown, using x-ray diffraction, to change its position following cross-bridge binding (38, 688, 689). Cooperative binding of myosin heads has been demonstrated in biochemical measurements and in studies of smooth muscle preparations (35, 101, 619, 696). This mechanism might be important for regulation attachment of phosphorylated and nonphosphorylated myosin to actin and can be further modulated by ADP binding to myosin (339, 340). Although these findings might also suggest the presence of thin filament Ca^{2+}-regulatory systems, no analog of the troponin in striated muscle has been found for smooth muscle.

Caldesmon is a calmodulin- and actin-binding protein that is found in the smooth muscle contractile apparatus (211, 419). It is bound to thin filaments and interacts with tropomyosin, myosin, and Ca^{2+}/calmodulin (445, 611). It has been proposed that caldesmon has an inhibitory role similar to that of troponin in striated muscle (cf. Ref. 446) or that it influences the myosin actin interaction (cf. Ref. 102). Although binding of caldesmon can influence the position of tropomyosin, these effects are not identical to those induced in striated muscle filaments by troponin (381). Caldesmon has an inhibitory function on actomyosin ATPase and can inhibit force at low phosphorylation levels in permeabilized smooth muscle (439, 546). Caldesmon might thus be important for cooperative regulation of cross-bridge attachment. It is also possible that caldesmon is regulated in the muscle cells. Ca^{2+}/calmodulin binding and caldesmon phosphorylation reverses its inhibitory function (500, 609). Caldesmon can be phosphorylated in vitro by several kinases including CaM kinase II (578), PKC (4), casein kinase II (703), ERK family of MAP kinases (119), and PAK (196, 684). Several stimuli lead to caldesmon phosphorylation in the muscle cells, but the main kinase(s) and the role of caldesmon phosphoryla-

tion in the regulation of contraction of intact smooth muscle tissue are not fully clear at present.

Calponin was first described by Takahashi et al. (638). This protein is associated with actin filaments, and it is localized in the contractile and cytoskeletal domains of the smooth muscle cell (507, 700). Calponin exists in different isoforms in smooth muscle (29, 639). It interacts with several contractile, cytoskeletal, and regulatory proteins (cf. Ref. 230) and inhibits actin myosin interaction in a tropomyosin-independent manner (719). Calponin has been proposed to have a role in the cytoskeleton possibly via cross-linking actin and intermediate filaments (230, 605). Calponin inhibits filament sliding in in vitro motility assay (70, 248, 308, 589) and inhibits contraction in smooth muscle preparations (308, 510), which shows that it also can influence the contractile system directly. Interestingly, calponin seems to mainly influence the V_{max} (308, 510). Consistent with this, ablation of the h1 (basic) calponin gene in smooth muscle was associated with increased shortening velocity and faster rate of tension development in smooth muscle tissues, including the urinary bladder (210, 456, 640). Calponin has also been found to translocate in the cell (530) and to interact with protein kinases (464), which could suggest a role for calponin in cell signaling possibly as an adapter protein. However, no alteration in Ca^{2+} regulation has been observed in calponin-deficient mice (456, 742). Calponin can be phosphorylated in vitro by several kinases including CaM kinase II, PKC and *Rho* kinase (321). However, the role of calponin phosphorylation in the intact muscle cell is less clear; no alteration in calponin phosphorylation following agonist activation has been reported (52).

V. MEMBRANE EXCITATION

A. Resting Membrane Potential and Action Potentials

Electrophysiological information on detrusor muscle has been obtained from several species, including humans (72, 75, 134, 135, 137, 204, 265, 266, 481–483, 492, 670, 691–694). In guinea pig detrusor tissue, resting membrane potential values from −31 to −53 mV have been reported (135, 265, 266, 692); the corresponding mean value in the rabbit was −37 mV (95), and in the rat −47 mV (138). In isolated human detrusor cells, the membrane potential, recorded by conventional 3 M KCl-filled microelectrodes, was found to be between −47 and −55 mV (471, 695). In, e.g., guinea pig and human freshly isolated detrusor cells, a membrane potential of about −60 mV was recorded (629). This value is more negative than those usually found in multicellular preparations (77). In cultured human and guinea pig detrusor cells, the value was around −40 mV (629).

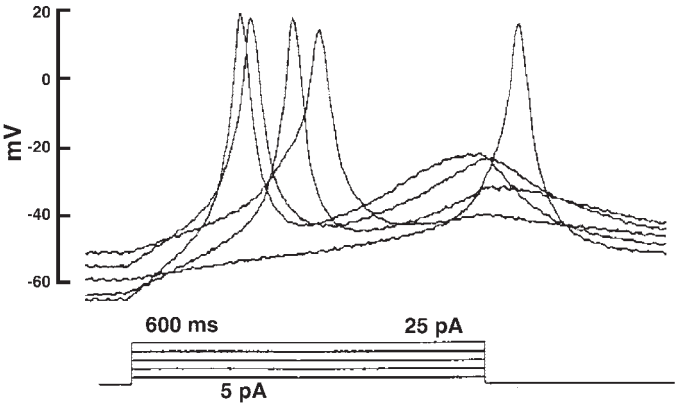

FIG. 3. Action potentials in human detrusor cells. [Modified from Sui et al. (629).]

Spontaneous action potentials, associated with slow waves of depolarization, have been demonstrated by several investigators in detrusor tissue from, e.g., guinea pig and rabbit (134, 135, 137, 265, 266, 481, 492, 670). Similar action potentials have been described in human detrusor cells (Fig. 3) (138, 471, 691, 695). The spontaneous electrical activity of the guinea pig detrusor is usually single spikes occurring at regular intervals, but occasionally bursts of spikes can be recorded (135, 265, 266). The frequency of the spontaneous action potentials is voltage sensitive, with depolarization increasing and hyperpolarization decreasing the rate of firing (481). Intracellularly recorded action potentials had rise times between 35.7 and 92.4 ms and half-widths between 8.3 and 10.5 ms (266). The peak amplitudes varied between 40.7 and 59.6 mV (266). In guinea pig detrusor, the action potentials exhibit a pronounced afterhyperpolarization, with amplitudes ranging between 5.6 and 16.7 mV (266, 481).

The rising phase of the action potential is generated by an inward Ca^{2+} current, as suggested by the failure of tetrodotoxin (TTX) (blocking Na^+ channels) to block elicited action potentials (134), the sensitivity to L-type calcium channel blockers (77, 265, 266, 481), and, as demonstrated (358), using voltage clamp of isolated guinea pig detrusor cells. Also in the human detrusor, the depolarizing phase of the action potential occurs by an inward Ca^{2+} current (471). Repolarization probably involves inactivation of the Ca^{2+} current and activation of an outward K^+ current that is partially Ca^{2+} dependent (302, 471, 481).

As shown in guinea pig bladder, the spontaneous action potentials are associated with a rise in $[Ca^{2+}]_i$; they are not prevented by caffeine, ryanodine, or cyclopiazonic acid, suggesting that intracellular Ca^{2+} stores do not contribute to their generation (265). However, Ca^{2+} release from intracellular stores may amplify the increase in $[Ca^{2+}]_i$ associated with the action potentials. The increases in $[Ca^{2+}]_i$ occurred along the boundary of smooth

muscle bundles and then propagated to other bundles through gap junctions (266).

Single transmural stimuli initiate excitatory junction potentials (EJPs) in guinea pig detrusor muscle cells, which trigger action potentials, associated with transient increases in $[Ca^{2+}]_i$ and associated contractions (265). Because the responses were abolished by α,β-methylene ATP, it was suggested that they resulted from the activation of purinoceptors by neurally released ATP (265). Nifedipine abolished the action potentials, leaving the underlying EJPs, and reduced the amplitude of both nerve-evoked increases in $[Ca^{2+}]_i$ and associated contractions. Subsequent coapplication of caffeine and ryanodine inhibited the residual responses without inhibiting EJPs (265). These results suggested that ATP, by activation of purinoceptors, initiates EJPs, which in turn trigger action potentials and influx of Ca^{2+} through L-type Ca^{2+}channels, and also Ca^{2+} release from intracellular Ca^{2+}stores. This suggestion is well in line with the results of previous investigators.

B. Ca^{2+} Channels

There is no doubt that an increase in $[Ca^{2+}]_i$ is a key process required for the activation of contraction in the detrusor. However, it is still discussed whether this increase is due to influx from the extracellular space and/or release from intracellular stores. Furthermore, the importance of each mechanism in different species, and also with respect to the transmitter studied, has not been established (319). Several types of Ca^{2+} channels have been demonstrated in smooth muscle (59), but for the detrusor, information is available mainly on voltage-operated channels. For example, in many types of smooth muscle, there are at least two, and probably more, distinct store-operated channels (SOCs), which have markedly different permeabilities to Ca^{2+} (7). They can be expected to occur also in the detrusor, but if they have any importance for detrusor contraction does not seem to have been specifically studied.

In single smooth muscle cells of the guinea pig urinary bladder, held under voltage clamp, Ganitkevich and Isenberg (218) recorded $[Ca^{2+}]_i$ by means of the Ca^{2+} indicator indo 1. They concluded that Ca^{2+} transport through voltage-operated Ca^{2+} channels is the key event in depolarization-mediated changes in $[Ca^{2+}]_i$. The voltage dependence of peak $[Ca^{2+}]_i$ was bell shaped, which supported the idea that $[Ca^{2+}]_i$ is controlled by Ca^{2+} influx through Ca^{2+} channels and that Ca^{2+} influx via the Na^+/Ca^{2+} exchanger is insignificant. They also concluded that depolarization-induced influx of Ca^{2+} through L-type Ca^{2+} channels induces a release of Ca^{2+} from intracellular stores, which constitutes a major portion of the phasic $[Ca^{2+}]_i$ transient. Imaizumi et al. (302), studying $[Ca^{2+}]_i$ transients and electrical events in guinea pig detrusor, concluded from their experiments that the entry of Ca^{2+} in the early stages of an action potential evokes Ca^{2+}-induced Ca^{2+} release from discrete subplasmalemma Ca^{2+} storage sites and generates "hot spots" close to the cell membrane that spread to initiate a contraction. This is in line with the findings of Hashitani et al. (266) mentioned above.

It has been shown that muscarinic receptor stimulation (carbachol) can suppress the L-type Ca^{2+} current in pig and human detrusor cells (319, 743). This was suggested to occur via two independent mechanisms: *1*) Ca^{2+}-mediated inactivation of the Ca^{2+} channel, caused by Ca^{2+} release from IP_3- and thapsigargin-sensitive internal stores, and *2*) a GTP-binding protein-mediated mechanism, which requires intracellular Ca^{2+} (743). However, based on their experiments, Kajioka et al. (319) suggested this suppression to occur irrespective of IP_3-induced Ca^{2+} release. Instead, it was attributed to involvement of a G protein-coupled mechanism (319). The role of this muscarinic receptor-mediated inhibitory action of L-type Ca^{2+} channels in the physiological regulation of detrusor contraction is still unknown.

When contracted by high-K^+ solutions or prolonged application of agonists, the detrusor muscle has an inability to sustain the contraction, and the response fades rapidly. Brading (72) suggested that this may be due to a transient increase in the membrane permeability to Ca^{2+}, the Ca^{2+} channels closing even at persistent depolarization. A well-developed Ca^{2+}-induced inactivation of the channels may account for the phasic nature of the detrusor contraction and its inability to sustain tone (494).

Several studies on animal detrusor tissue have demonstrated the importance of extracellular Ca^{2+} entry through dihydropyridine-sensitive Ca^{2+} channels and mobilization of intracellular Ca^{2+} for activation of the detrusor via the main transmitters, acetylcholine and ATP (see Ref. 18). In addition, muscarinic receptor stimulation was suggested to increase the sensitivity of the contractile machinery to Ca^{2+} (351). The relative contribution of these mechanisms to activation of contration has not been established. In the human detrusor (197), extracellular Ca^{2+} seems to play a major role in the activation process. However, the contribution of intracellular store release and extracellular influx to muscarinic receptor stimulation often differs between studies (55, 261, 350, 449, 693, 723). Several studies have demonstrated an importance of extracellular Ca^{2+} entry in the contraction induced by muscarinic and/or purinergic stimulation; the most common entry pathway is through dihydropyridine-sensitive Ca^{2+} channels. Uchida et al. (667) suggested that the tonic-contractile response induced by carbachol in pig detrusor smooth muscle strips was dependent mainly on depolarization of the cell membrane and an influx of extracellular Ca^{2+} and that the depolarizing response may

be due to inactivation of ATP-sensitive K^+ channels through muscarinic receptor activation of PKC. Wu et al. (726) suggested that refilling of intracellular Ca^{2+} stores occurs via Ca^{2+} influx through L-type Ca^{2+} channels and that Ca^{2+} influx is regulated by a feedback mechanism whereby a fall of $[Ca^{2+}]_i$ reduces the activity of Ca^{2+}-activated K^+ channels, causing cell depolarization and an enhancement of L-type Ca^{2+} channel conductance.

The role of other Ca^{2+} channels than the L-type in detrusor activation has been debated. In the human detrusor, Maggi et al. (428) found that different agonists used different Ca^{2+} sources to various degrees, and suggested that carbachol-induced activation involved dihydropyridine-sensitive Ca^{2+} channels, but not T- or N-type channels. Thus, in human and guinea pig detrusor cells, the contribution of Ca^{2+} influx via T-type channels has been considered of no importance (305, 428). However, Sui et al. (628) found that in cultured detrusor cells, the action potential was completely abolished by L-type Ca^{2+} channel blockers, but incompletely so in freshly isolated cells. Outward current depended strongly on previous inward current, suggesting a predominant Ca^{2+}-dependent outward current. They concluded that in freshly isolated guinea pig cells, T- and L-type Ca^{2+} currents are present, but T-type current is absent in confluent cultures. Chow et al. (121) found that in normal extracellular solution $NiCl_2$ and verapamil reduced electrically stimulated contractions and concluded that the data were consistent with the hypothesis that Ca^{2+} influx through both T-type and L-type Ca^{2+} channels determines the contractile status of detrusor smooth muscle and that T-type channel activity is more important at membrane potentials near the resting level. A significant role for T-type channel activity in the resting state was evident in that spontaneous contractions were attenuated to a greater extent than evoked contractions by the blocking agents used (121).

C. K^+ Channels

Among the many K^+ channels that have been described (716), the importance for detrusor function of ATP-sensitive and Ca^{2+}-activated (large, intermediate, and small conductance) channels has been established. Information on the occurrence and function of other K^+ channels (including voltage-gated K^+ channels) in the detrusor is scarce.

1. ATP-sensitive K^+ channels

Urinary bladder smooth muscle expresses ATP-sensitive K^+ (K_{ATP}) channels, which have been suggested to be involved in the regulation of bladder contractility (17, 67), and the presence of mRNA for sulfonylurea receptors has been demonstrated in both pig and human detrusor (84). As mentioned previously, the frequency of spontaneous action potentials is voltage sensitive. Therefore, hyperpolarization of the detrusor muscle cell membrane can decrease action potential firing and associated contraction. K^+ channel openers, e.g., cromakalim, pinacidil, and nicorandil ("first generation"), produce hyperpolarization by the opening of K^+ channels and subsequent efflux of K^+ (see Refs. 17, 18). Cromakalim was shown to reduce the spike frequency in isolated guinea pig detrusor muscle, and at high concentrations (10^{-6} to 10^{-5} M), it abolished the spikes and produced concentration-dependent hyperpolarization of the cell membrane (194, 195). Spontaneous contractile activity was abolished. Similar effects have been demonstrated with pinacidil (581). Cromakalim opened a K^+ channel having properties similar to those of the delayed rectifier K^+ channel responsible for spike repolarization. This channel is similar to the ATP-dependent K^+ channels in vascular smooth muscle (208).

A critical issue in understanding the role of K_{ATP} channels is the relationship between channel activation and the effect on tissue function. Petkov et al. (541) explored this relationship in guinea pig detrusor cells by activating K_{ATP} channels with the compounds *N*-(4-benzoylphenyl)-3,3,3-trifluoro-2-hydroxy-2-methylpropionamide (ZD-6169) and levcromakalim. ZD-6169 and levcromakalim caused half-maximal activation ($K_{1/2}$) of K_{ATP} currents in single cells at 1.02 and 2.63 μM, respectively. In contrast, much lower concentrations ($K_{1/2}$ = 47 nM for ZD-6169 and $K_{1/2}$ = 38 nM for levcromakalim) caused inhibition of action potentials and phasic contractions of the muscle cells. The results suggested that activation of <1% of K_{ATP} channels is sufficient to inhibit significantly action potentials and the related phasic contractions.

Studies on isolated detrusor muscle from humans and several animal species (see Ref. 18) have shown that K_{ATP} channel openers reduce not only spontaneous contractions, but also contractions induced by electrical stimulation, carbachol, and low, but not high, external K^+ concentrations. However, e.g., cromakalim was found to abolish spontaneous activity, but not to prevent maximum activation of the detrusor through various different pathways, including nerve stimulation (195). The drugs also increase the outflow of ^{86}Rb or ^{42}K in preloaded tissues, further supporting the view that they relax bladder tissue by K_{ATP} channel opening and subsequent hyperpolarization. The K_{ATP} channel openers were particularly effective in hypertrophic rat bladder muscle in vitro (433), and effectively suppressed detrusor overactivity in rats with bladder outflow obstruction (434). However, the K_{ATP} channel openers used were approximately eight times more potent as inhibitors of the spontaneous contractions of the rat portal vein than of K^+-induced contractions of the rat detrusor (167). Selectivity problems

have limited the use of K_{ATP} channel openers in the treatment of detrusor overactivity (21).

2. Ca^{2+}-activated K^+ channels

In guinea pig detrusor cells, Grant and Zuzack (242) found marked stimulatory effects of charybdotoxin and iberiotoxin, which were used as probes for investigation of the large-conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels, and suggested that these channels may be involved in the control of basal tension and possibly the membrane potential. That the BK_{Ca} channels play an important role in controlling membrane potential and contractility of detrusor smooth muscle has been confirmed by other investigators (276, 325). These channels are composed of a pore-forming α-subunit and an accessory, smooth muscle-specific, β_1-subunit. Petkov et al. (540) determined the functional role of the β_1-subunit in controlling the contractions of detrusor by using β_1-subunit knock-out (KO) mice. They found that the β_1-subunit is highly expressed in normal detrusor smooth muscle. In the BK_{Ca} channels lacking β_1-subunits, the activity was reduced, with a shift in voltage/Ca^{2+} sensitivity. Iberiotoxin increased the amplitude and decreased the frequency of phasic contractions of detrusor strips from control mice. The detrusor strips from β_1-subunit KO mice had elevated phasic contraction amplitude and decreased frequency compared with control detrusor strips. Iberiotoxin increased the amplitude and frequency of phasic contractions and tone of detrusor strips from β_1-subunit KO mice, suggesting that BK_{Ca} channels still regulate contractions in the absence of the β_1-subunit. The results indicated that the β_1-subunit, by modulating BK_{Ca} channel activity, plays a significant role in the regulation of phasic contractions of the detrusor.

Measurements of BK_{Ca} currents and intracellular Ca^{2+} have revealed that BK_{Ca} currents are activated by Ca^{2+} release events (Ca^{2+} sparks) from ryanodine receptors on the SR (278). Such receptors (189) have been demonstrated in human detrusor (104). Herrera et al. (278) characterized Ca^{2+} sparks and BK_{Ca} currents and determined the voltage dependence of the coupling of ryanodine receptors to BK_{Ca} channels in guinea pig detrusor smooth muscle. Ca^{2+} sparks in this muscle had properties similar to those described in arterial smooth muscle. Most Ca^{2+} sparks caused BK_{Ca} currents at all voltages tested, consistent with the BK_{Ca} channels sensing $\sim$10 μM Ca^{2+}. Membrane potential depolarization from -50 to -20 mV increased Ca^{2+} spark and BK_{Ca} current frequency threefold. However, membrane depolarization over this range had a differential effect on spark and current amplitude, with Ca^{2+} spark amplitude increasing by only 30% and BK_{Ca} current amplitude increasing 16-fold. A major component of the amplitude modulation of spark-activated BK_{Ca} current was quantitatively explained by the known voltage dependence of the Ca^{2+} sensitivity of BK_{Ca} channels. Herrera et al. (278) proposed that membrane potential, or any other factor that modulates the Ca^{2+} sensitivity of BK_{Ca} channels, profoundly alters the coupling strength of Ca^{2+} sparks to BK_{Ca} channels. In the guinea pig detrusor, Ohi et al. (511) found that a limited number of discrete SR fragments in the subplasmalemmal area seemed to play key roles in the control of BK_{Ca} channel activity by generating Ca^{2+} sparks at rest to activate spontaneous transient outward currents (STOCs) and by generating Ca^{2+} transients presumably triggered by sparks during an action potential.

Not only BK_{Ca} channels but also small-conductance (SK_{Ca}) channels are regulators of excitability in detrusor smooth muscle. Herrera and Nelson (279) examined the role of SK_{Ca} channels in guinea pig detrusor, using the SK_{Ca} channel blocker apamin. They found that apamin caused a concentration-dependent increase in the amplitude of phasic contractions over a broad concentration range (10^{-10} to 10^{-6} M). To determine the effects of Ca^{2+} signals from voltage-dependent Ca^{2+} channels and ryanodine receptors to SK_{Ca} and BK_{Ca} channels, Herrera and Nelson (279) measured whole cell membrane current in isolated myocytes bathed in physiological solutions. Their results indicated that Ca^{2+} entry through voltage-dependent Ca^{2+} channels activates both BK_{Ca} and SK_{Ca} channels, but Ca^{2+} release (Ca^{2+} sparks) through ryanodine receptors activates only BK_{Ca} channels.

Christ et al. (124) demonstrated the importance of the BK_{Ca} channel, using local hSlo cDNA (i.e., the BK_{Ca} channel) injection to ameliorate detrusor overactivity in a rat model of partial urinary outlet obstruction. They found that saline-injected obstructed rats routinely displayed spontaneous detrusor contractions between micturitions. In contrast, hSlo injection eliminated the obstruction-associated detrusor overactivity, without detectably affecting any other cystometric parameter. Christ et al. (124) suggested that expression of hSlo in rat bladder functionally antagonized the increased contractility normally observed in obstructed animals and thereby ameliorated detrusor overactivity. They also thought that their observations could indicate a potential utility of gene therapy for urinary incontinence.

3. K_v channels

In the mouse detrusor myocytes, Thorneloe and Nelson (655) characterized the biophysical, pharmacological, and molecular properties of the voltage-gated K^+ current and suggested that these heteromultimeric channels could be potential targets for modulation of detrusor function. Davies et al. (145) determined whether voltage-gated K^+ (K_V) channels are expressed and functional in human detrusor smooth muscle. They obtained information by using electrophysiological patch clamp, immuno-

fluorescence, Western blot, and isometric tension recording techniques. Patch-clamp recordings from detrusor cells revealed a Ca^{2+}-independent K^+ current that was activated by depolarization in a voltage range near the resting potential of detrusor smooth muscle. The current was inhibited by 3,4-diaminopyridine, a blocker of K_V channels. Antibodies targeted to $K_{V\alpha1}$ subunits revealed $K_{V1.3}$ and $K_{V1.6}$ expression in whole bladder tissue samples and specifically in detrusor smooth muscle cells. Blockers of $K_{V\alpha1}$ channel currents (correolide and recombinant agitoxin-2) had a myogenic effect, characterized by increased amplitude of spontaneous contractions without an effect on the frequency of contractions or on resting baseline tension. Davies et al. (145) concluded that $K_{V\alpha1}$ subunits are expressed and functionally important in human detrusor muscle. Taken together, available information suggests the occurrence of K_V channels in the detrusor. The importance of these channels for detrusor function in vivo remains to be studied.

D. Stretch-Activated Channels

In detrusor muscle cells, mechanical stretch of the cell membrane has been suggested to activate nonspecific cation channels (708–711). The channels show similar permeabilities to Na^+, K^+, and Ca^{2+}, and the open probability is enhanced after prior hyperpolarization. The channels can be blocked by the gadolinium ion (Gd^{3+}) and by grammostola spatulata venom (93, 652). If a quiescent cell is stretched by >20% of its resting length, the Ca^{2+} influx through the channel is sufficient to raise the $[Ca^{2+}]_i$ significantly, and the total current will be enough to depolarize the cell sufficiently to open L-type Ca^{2+} channels, and provoke Ca^{2+} influx (204). As pointed out previously (204), stretch-activated channels have the potential to act as length detectors in the bladder wall.

Ji et al. (312) demonstrated that in isolated cells from animal detrusors (rabbit and mouse), increases in cell length resulted in the gating of ryanodine receptors and the release of Ca^{2+} from the SR in the form of Ca^{2+} sparks or propagated Ca^{2+} waves. The release was not affected by IP_3 receptor-mediated Ca^{2+} release, but was completely blocked by ryanodine. Interestingly, the release evoked Ca^{2+}-activated Cl^- currents, suggesting a regulatory mechanism for the generation of spontaneous currents in the cells.

Hyposmotically induced cell swelling has been commonly used to stimulate stretch-activated channels in various cell models, even if this approach has been criticized (552). Chambers et al. (103) showed that isolated human detrusor smooth muscle cells are sensitive to membrane stretch by hyposmotic solutions and that the mechanisms involved include the direct mobilization of intracellular Ca^{2+}. They also suggested that there may be differences in the responsiveness of cells isolated from stable and unstable (overactive) bladders. Using a similar model, Tertyshnikova et al. (652) showed that gadolinium and grammostola spatulata venom inhibited stretch-induced calcium signaling in human detrusor myocytes. The venom improved bladder compliance and inhibited the frequency of spontaneous bladder contractions in an in vitro model of rat bladder. Based on their results, they suggested that activation of stretch-induced signaling in detrusor cells may have an important role in myogenic regulation of detrusor contractility during bladder filling and that inhibition of stretch-activated channels may improve bladder compliance.

Kushida et al. (367) investigated stretch-induced signaling by studying the MAP kinase family using rat primary detrusor smoth muscle cells. Sustained mechanical stretch using collagen-coated silicon membranes caused activation of c-Jun NH_2-terminal kinase (JNK). Activation of p38 was weak compared with that of JNK, and extracellular signal-regulated kinase (ERK) was not activated at all. JNK activation by mechanical stretch was totally dependent on extracellular $Ca^{2+}$and inhibited by Gd^{3+}. Nifedipine and verapamil had no effect on this JNK activation. Moreover, none of the inhibitors pertussis toxin, genistein, wortmannin, or calphostin C affected stretch-induced JNK activation, indicating that G protein-coupled and tyrosine kinase receptors are unlikely to be involved in this JNK activation. On the other hand, W-7, a calmodulin inhibitor, and cyclosporin A, a calcineurin inhibitor, prevented JNK activation by stretch. The results of Kushida et al. (367) suggested a novel pathway for stretch-induced activation of JNK in detrusor cells: mechanical stretch evokes Ca^{2+} influx via Gd^{3+}-sensitive stretch-activated Ca^{2+} channels, resulting in JNK activation under regulation in part by calmodulin and calcineurin.

E. Ligand-Activated Channels

Ligand-gated ion channels occur in a number of cells and utilize a number of transmitters/modulators. In the detrusor muscle, ACh and ATP are of immediate interest. Activation of P2X receptors opens a channel, which is relatively nonselective to cations. This generates an inward current carried mainly by Na^+ and Ca^{2+}, leading to depolarization, which if sufficient to activate L-type Ca^{2+} channels will generate an action potential (304). Evans et al. (183) recorded membrane currents evoked by ATP using whole cell patch clamp in cells expressing $P2X_1$ and $P2X_2$ receptors. There was no difference between the two receptors with respect to their permeability to monovalent organic cations. The calcium permeability of $P2X_1$ receptors was greater than that of $P2X_2$ receptors. ATP-evoked currents in cells expressing the $P2X_2$ receptor

were strongly inhibited when the extracellular Ca^{2+} concentration was increased; the action of ATP could be restored by increasing the ATP concentration. ATP-evoked currents in cells expressing the $P2X_1$ receptor were not inhibited by such increases in the extracellular Ca^{2+} concentration.

F. Myogenic Activity

Myogenic activity, defined as the ability of a smooth muscle cell to generate contractile activity independent of external stimuli, is an important characteristic of detrusor smooth muscle. Even if classical gap junctions have been difficult to demonstrate in the human bladder, detrusor smooth muscle cells are believed to be electrically coupled (18, 143, 151). However, the nature of the underlying cell-cell communication has not been clearly demonstrated. Wang et al. (701) used double whole cell patch-clamp recordings together with Northern and Western blot techniques to characterize gap junction channel properties, and to evaluate connexin43 (Cx43) expression in human detrusor cells. The presence of Cx43 mRNA and protein in cultured detrusor myocytes, and also in situ, was demonstrated. Wang et al. (701) found human detrusor cells to be well coupled and concluded that gap junctions could have an important role in the initiation, maintenance, and modulation of detrusor tone. Neuhaus et al. (498) also used Western blot technique to demonstrate Cx43 in human bladder musculature and in smooth muscle cell cultures. They found expression of Cx43 in all detrusor samples investigated, and by iontophoretic application of gap junction-permeable fluorescent dye they also demonstrated cell-cell coupling in cultured smooth muscle cells. John et al. (317) found morphological, molecular biological, and immunohistochemical evidence that the smooth muscle cells of the normal human detrusor are electrically coupled through gap junctions. Ultrastructural and freeze-fracture studies showed gap junctions at detrusor smooth muscle cells. These gap junctions appeared to be small and irregularly shaped. RT-PCR and in situ hybridization showed that Cx45 mRNA was expressed in the detrusor muscle. Furthermore, prominent Cx45 immunoreaction was detected at detrusor smooth muscle cells. Freeze-fracture immunolabeling techniques revealed Cx45 signals at small gap junctional plaques.

Taken together, there is good evidence for gap junction coupling between human detrusor cells. Also in the trigonal smooth muscle, there is evidence for the occurrence of gap junctions (316). The presence of gap junctions, in concert with the autonomic nervous system and myogenic intracellular signal transduction mechanisms, is postulated to form a "syncytial tissue triad" that is largely responsible for the local modulation of smooth muscle tone (325). Drake and co-workers (157, 159) proposed that the detrusor muscle is arranged into modules, which are circumscribed areas of muscle during the filling phase of the micturition cycle. These modules might be controlled by a peripheral myovesical plexus, consisting of intramural ganglia and interstitial cells. Intercellular connections may contribute to module control.

Detrusor smooth muscle exhibits spontaneous rhythmic activity both in vivo and in vitro (18, 72, 157, 159, 277). The frequency of the spontaneous mechanical activity of isolated detrusor tissue seems to vary between species (590), being more frequent in the rabbit than in pig and human, but is probably also dependent on experimental factors, e.g., the length of the equilibration period in the organ bath. The characteristics of spontaneous contractile activity have been studied in detail in detrusor tissue from pigs (252, 682, 683) and rabbits (551).

The spontaneous electrical activity demonstrated in vitro is associated with contractions that are resistant to TTX and cannot be blocked by hexamethonium, atropine, α-adrenoceptor blockers, β-adrenoceptor blockers, or suramin, suggesting a myogenic origin (18, 300, 301). Contractions can be effectively inhibited by L-type Ca^{2+} channel blockers and K^+ channel openers (23, 198, 300, 301). They can also be increased by agents that decrease K^+ permeability (300, 301, 481). The effects of different types of K^+ channel modulators on the spontaneous rhythmic contractile activity were examined in guinea pig urinary bladder smooth muscle by Imai et al. (300). Guinea pig cells exhibited myogenic rhythmic contraction in the presence of atropine, phentolamine, propranolol, suramin, and TTX. Nisoldipine or diltiazem substantially diminished the contractile activity. A nisoldipine-resistant component of rhythmic contraction was further inhibited by Gd^{3+}. Iberiotoxin dramatically increased both contraction amplitude and frequency, whereas NS-1619, which increases BK_{Ca} channel activity, decreased them. Apamin increased contraction amplitude, but decreased frequency. 4-Aminopyridine, a blocker of K_V channels, significantly increased contraction frequency. E-4031, a blocker of an inwardly rectifying K^+ channel, i.e., the human ether-a-go-go-related gene (HERG) K^+ channel, significantly increased contraction amplitude. Glibenclamide and Ba^{2+} had little effects on the spontaneous contractile activity. These findings imply that BK_{Ca} and SK_{Ca} channels have prominent roles as negative-feedback elements to limit extracellular Ca^{2+} influx-mediated guinea pig detrusor contraction by regulating both amplitude and frequency. It was also suggested that both non-K_{Ca} type of K^+ (K_V and HERG-like K^+) channels may contribute to the regulation of myogenic rhythmic contraction.

Raising extracellular Mg^{2+} reduced spontaneous contractile activity and attenuated the inward Ca^{2+} current associated with the action potential. Spontane-

ous contractile activity was suggested to be related to generation of spontaneous action potentials, or to alteration of the ability of intracellular organelles to regulate intracellular calcium (471). Wibberley et al. (715) found that Rho-kinase inhibitors caused a significant decrease in baseline tension of isolated rat bladder strips, suggesting an involvement of the Rho-kinase pathway in myogenic tone.

Bladder outflow obstruction is often associated with increased spontaneous detrusor activity in vitro, and with detrusor overactivity in vivo. Bladder outflow obstruction is characterized by morphological bladder changes, including a progressive denervation and hypertrophy of the bladder wall (241, 385, 390, 391). An increase in tension and/or strain on the bladder, as in bladder outflow obstruction, has been shown to be associated with cellular and molecular alterations, e.g., in cytoskeletal and contractile proteins, in mitochondrial function, and in various enzyme activities of the smooth muscle cells (see sect. III*F*). Furthermore, the bladder has numerous interstitial cells (607), whose role in normal as well as hypertrophic bladders remains to be explained. Turner and Brading (666) suggested that in the overactive detrusor, alterations of the smooth muscle are seen, which may be a consequence of "patchy denervation" of the detrusor. This was supported by studies on human overactive bladders, in which areas of denervation were demonstrated, and the changes were suggested to be factors contributing to detrusor overactivity (106, 465). Elbadawi et al. (175) reported that in the overactive human bladder, obtained from geriatric patients, protrusion junctions and ultraclose cell abutments could be demonstrated as intercellular connections between detrusor muscle cells. They suggested that these connections may be mediators of the electrical coupling of muscle cells in detrusor overactivity. Other investigators, using animal models, have shown that there is an upregulation of Cx43 after experimental bladder outflow obstruction (122, 123, 249). The structural basis of neurogenic bladder dysfunction was recently investigated (250). Intermediate junctions of muscle cells were absent or reduced and instead intimate cell appositions with much narrower junctional gaps dominated. More than two intimate cell apposition-to-intermediate junction ratios were present in 98% of biopsies, and intimate cell apposition linked chains of five muscle cells or greater in all biopsies.

Changes in detrusor morphology, such as those occurring in the hypertrophic detrusor, may thus change cell-to-cell transmission of excitation. Seki et al. (580, 582) studied the changes in electrical properties of guinea pig smooth muscle membrane by experimental bladder outflow obstruction and found significant changes. There was a reduction of the membrane constants (space constant and time constant), and of spontaneously occurring electrical activity. The membrane potential was unchanged, but there was a greater amount of ouabain-sensitive membrane hyperpolarization after application of K^+-free solution and a greater membrane depolarization evoked by low extracellular Cl^- solution. It was suggested that there was a suppression of the cell-to-cell transfer of electrical activity and activation of a membrane electrogenic Na^+-K^+ pump mechanism in the obstructed and hypertrophied guinea pig detrusor.

Myogenic activity may be exaggerated in conditions where an increased afferent activity leads to conditions of detrusor overactivity. Thus Kinder and Mundy (348) found that spontaneous contractile activity developed more often in muscle strips from overactive than normal bladders. "Unstable" bladder contractions are often associated with hyperexcitability and changes in the nature of spontaneous phasic activity of the detrusor smooth muscle. Buckner et al. (85) characterized the spontaneous activity of the pig detrusor. They demonstrated that phasic activity was myogenic and was influenced by the presence of urothelium. Denuded strips exhibited robust spontaneous activity measured as mean area under the contraction curve compared with intact strips. Spontaneous phasic activity, particularly the amplitude, was dependent on both calcium entry through voltage-dependent Ca^{2+} channels and release from ryanodine receptors, as shown by inhibition of spontaneous activity by nifedipine and ryanodine, respectively. Inhibition of BK_{Ca} channels by iberiotoxin resulted in an increase in contraction amplitude and frequency. Apamin also increased contraction amplitude and frequency, demonstrating that these mechanisms are critical to the regulation of phasic spontaneous activity. Inhibition of K_{ATP} channels by glyburide did not significantly alter myogenic contractions. However, K_{ATP} channel openers effectively suppressed spontaneous myogenic activity. In general, they were 15-fold more potent in suppressing spontaneous activity than in inhibiting contractions evoked by electrical field stimulation. These studies suggested that modulation of K^+ channels, particularly K_{ATP} channels, may offer a unique mechanism for controlling spontaneous myogenic activity, especially those associated with the hyperexcitability occurring in overactive bladders.

G. Interstitial Cells

Spindle-shaped cells, where cGMP immunoreactivity can be induced, have been found in urinary bladders of guinea pig and humans (607). Smet et al. (607) found the cells present in large amounts throughout the muscle bundles and within the interstitium of the bladder, but that they were particularly concentrated in the outer fibromuscular coat, where the processes formed a dense

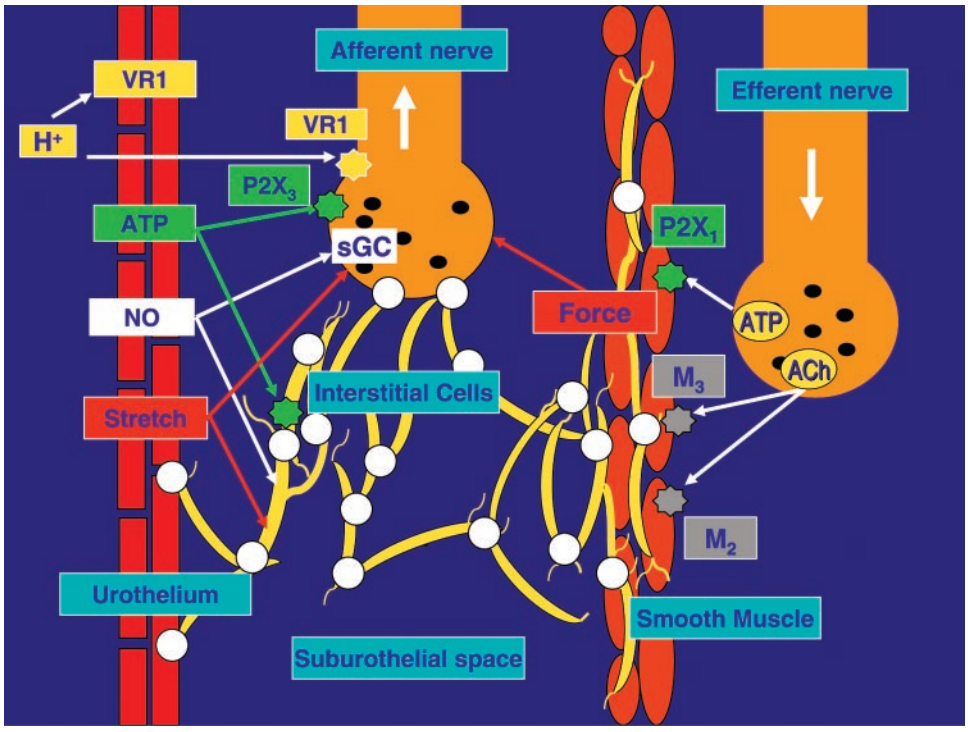

FIG. 4. Hypothetical schematic model of urothelial signaling and possible role of interstitial cells for detrusor activity. Stretch of the bladder during filling may evoke activity in afferent nerves directly or via interstitial cells. The interstitial cells may also mediate signals from other agents generated by and released from the urothelium (ATP: $P2X_3$ receptors; NO: soluble guanylyl cyclase, sGC). Stimulation of vanilloid receptors (VR1) on the urothelium, afferent nerves, and/or interstitial cells may also have effects on the detrusor. Afferent nerves register changes in smooth muscle cells (force) induced by stretch, myogenic activity (contraction-relaxation), or transmitters (ATP: $P2X_1$; acetylcholine, ACh: muscarinic M_2 and M_3 receptors).

interconnecting network. c-*kit*-positive cells, similar to the interstitial cells of Cajal, have also been demonstrated throughout the guinea pig urinary bladder, located in parallel with the smooth muscle cells (461). This is in contrast to the findings of Pezzone et al. (543) in the mouse. They found no c-*kit*-positive interstitial cells in the bladder, only in the lamina propria of the ureteral pelvis. McCloskey and Gurney (461) demonstrated that the guinea pig insterstitial cells were spontaneously active and that they reacted to muscarinic receptor stimulation with firing of Ca^{2+} waves. They suggested that the cells could act as pacemakers or intermediaries in the transmission of nerve signals to smooth muscle cells. Supporting such a function, Sui et al. (627) found prominent labeling for the gap junction protein Cx43 in a suburothelial band of interstitial cells in the human bladder. Sui et al. (627) concluded that this network of suburothelial interstitial cells in human bladder may provide pathways for direct cell-to-cell communication and that the interstitial cellular network may operate as a functional syncytium, integrating signals and responses in the bladder wall. Drake et al. (158), on the other hand, confirmed the finding of Smet et al. (607) that the human detrusor possessed an extensive network of stellate fibroblastic cells located peripherally on muscle fascicles and suggested that they may be important for the secretion and maintenance of extracellular material. Wiseman et al. (720) described in detail a layer of cells located within the lamina propria of the human bladder and found that they had characteristics of both fibroblasts and smooth muscle. These cells had close contacts with nerves, apparently both afferent and efferent, and they were suggested to function as bladder stretch receptors. Vimentin-positive, smooth muscle α-actin-negative cells expressing NM-MHC-B have been reported to be present in the urinary bladder interstitium (408, 599). The NM-MHC-B is expressed in intestinal Cajal cells (570), which could suggest that the NM-MHC-B expressing cells of the bladder are identical with the Cajal-like cells in the bladder. Because the NM-MHC-B cells increase in number during bladder hypertrophy (599), it can be speculated that these cells contribute to alterations in the contractile activity of the bladder during hypertrophy. Obviously, interstitial cells with similarities to the Cajal cells of the gut can be demonstrated both suburothelially and within the detrusor muscle of both animal and human bladders. However, it is unclear whether or not these cells represent more than one population, and so far their functional roles have not been established.

Signals from the urothelium may be important not only for the development of the detrusor (54), but also for its contraction and relaxation in the adult individual. Thus it has been suggested that an unknown relaxant factor, released from the urothelium, can produce detrusor relaxation (270). It cannot be excluded that if this mechanism is physiologically important, e.g., during bladder filling, interstitial cells may be important for the communication between urothelium and detrusor smooth muscle (Fig. 4).

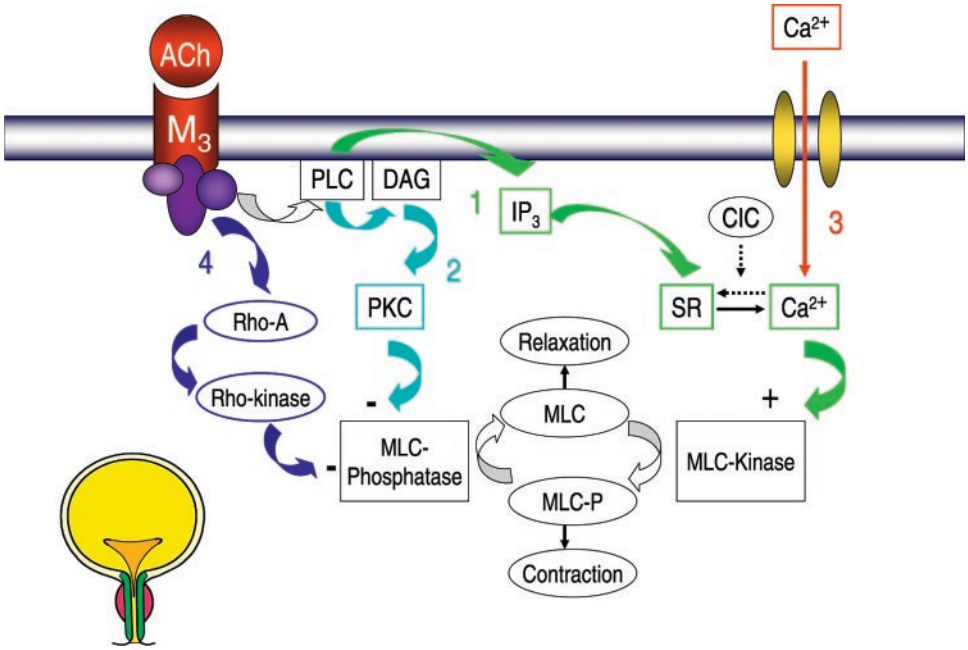

FIG. 5. Signal pathways (*1–4*) involved in activation of detrusor contraction via muscarinic M_3 receptors. ACh, acetylcholine; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; MLC, myosin light chain; IP_3, inositol trisphosphate; SR, sarcoplasmic reticulum; CIC, calcium-induced calcium release. There seem to be differences between species in the contribution of the different pathways in contractile activation. In human detrusor, Ca^{2+} influx (*3*) is of major importance.

VI. NEURAL AND HORMONAL CONTROL

A. Cholinergic Mechanisms

1. Muscarinic receptors

In most animal species, bladder contraction is mediated by both cholinergic and nonadrenergic, noncholinergic (NANC) mechanisms (14, 637). In isolated guinea pig and rabbit detrusor muscle, acetylcholine produced slight depolarization, initiated spike generation, increased the frequency of action potentials, and contracted the muscle (94, 135, 137). Also, the isolated human detrusor is contracted by acetylcholine. These contractions are enhanced by cholinesterase inhibitors and abolished by atropine, and thus mediated by stimulation of muscarinic receptors. In the normal human detrusor, the emptying contraction in vivo and the contraction evoked by electrical stimulation of nerves in vitro has been suggested to be mediated mainly, if not exclusively, through muscarinic receptor stimulation (56, 347, 515, 522, 590, 596, 636), because these responses can be more or less completely blocked by atropine.

Detrusor smooth muscle from various species contains muscarinic receptors of the M_2 and M_3 subtypes (117, 168, 272). In the human bladder, the occurrence of mRNAs for all muscarinic receptor subtypes has been demonstrated (594), with a predominance of mRNAs encoding M_2 and M_3 receptors (594, 733). These receptors are also functionally coupled (117, 188, 272). Muscarinic receptors are coupled to G proteins, but the signal transduction systems vary (99). M_1, M_3, and M_5 receptors couple preferentially to $G_{q/11}$, activating phosphoinositide hydrolysis, in turn leading to mobilization of intracellular calcium. M_2 and M_4 receptors couple to pertussis toxin-sensitive $G_{i/o}$, resulting in inhibition of adenylyl cyclase activity.

Jezior et al. (311), working with rabbit detrusor, found that bethanechol-induced contractions were practically abolished by inhibitors of Rho-kinase (Y27632, HA 1077) in combination with a nonselective cation channel inhibitor (LOE-908). They suggested that muscarinic receptor activation of detrusor muscle includes both nonselective cation channels and activation of Rho-kinase. Supporting a role of Rho-kinase in the regulation of rat detrusor contraction and tone, Wibberley et al. (715) found that Rho-kinase inhibitors (Y-27632, HA 1077) inhibited contractions evoked by carbachol without affecting contraction to KCl. They also demonstrated high levels of Rho-kinase isoforms (I and II) in the bladder. The main signaling pathways involved in muscarinic (M_3-receptor) activation of the detrusor are depicted in Figure 5.

In cat detrusor muscle, contraction induced by acetylcholine was found to be mediated via M_3 receptor-dependent activation of $G_{q/11}$ and PLC-β_1 and IP_3-dependent Ca^{2+} release (15) (Fig. 5). Also in the human bladder the muscarinic receptors are believed to cause a direct smooth muscle contraction through phosphoinositide hydrolysis (25, 261). However, in most studies of the contribution of IP_3 production to muscarinic receptor-mediated contractions in the detrusor, relatively high concentrations of muscarinic receptor agonists have been used (25, 293, 480). It may be that the concentration of neurally released acetylcholine, which acts on the muscarinic receptors of the detrusor, is not always sufficiently high to stimulate IP_3 production. As suggested by Hashitani et al.

(265), muscarinic receptors, which stimulate the production of IP_3, may operate at high concentrations of muscarinic agonist, whereas M_2 muscarinic receptors, which do not trigger the formation of IP_3, may be activated by lower concentrations of agonist. Alternatively, bladder smooth muscle might have a difference in the localization of muscarinic receptors, in that junctional receptors may be M_2 receptors, and while extrajunctional receptors are M_3 receptors (265).

The signaling mechanisms for the M_2 receptors are less clear than those for M_3 receptors. It has been suggested that M_2 receptors may oppose sympathetically mediated smooth muscle relaxation, mediated by β-adrenoreceptors via inhibition of adenylyl cyclase (271). In agreement with this, Matsui et al. (452) suggested, based on results obtained in M_2 receptor KO mice, that a component of the contractile response to muscarinic agonists in smooth muscle involves an M_2 receptor-mediated inhibition of the relaxant effects of agents that increase cAMP levels. M_2 receptors may exert a modulatory effect on purine-evoked relaxations, as was shown in rat urinary bladder (227).

M_2 receptor stimulation can also activate nonspecific cation channels (361) and inhibit K_{ATP} channels through activation of PKC (68). Nakamura et al. (493) studied the functional relationship between the M_2 receptor and the Ca^{2+}-activated K^+ channel, by investigating the effect of carbachol on the membrane current of rat bladder smooth muscle cells. They found that carbachol induced a transient outward current via Ca^{2+} release from the SR (mediated through M_3 receptors and IP_3). This activates BK_{Ca} channels, which are then inhibited by a M_2 receptor and G_i-mediated signal transduction pathway. It was suggested that this M_2 receptor-mediated pathway enhances contraction, which is initiated by M_3 stimulation in rat bladder smooth muscle.

There is general agreement that M_3 receptors are mainly responsible for the normal micturition contraction (117, 120, 188, 272). Even in the obstructed rat bladder, M_3 receptors were found to play a predominant role in mediating detrusor contraction (364). The functional role of the M_2 receptor in the normal detrusor is less clear. In M_2 receptor KO mice, the bladder response to carbachol both in vitro and in vivo was practically unaltered (298), and in mice lacking the M_3 receptor, M_2 receptors mediated only ~5% of the response to carbachol (453, 454, 623). On the other hand, in certain disease states, M_2 receptors may contribute to contraction of the bladder. Thus, in the denervated rat bladder, M_2 receptors or a combination of M_2 and M_3 mediated contractile responses (78, 79).

As indicated above, the muscarinic receptor functions may be changed in different urological disorders, such as outflow obstruction, neurogenic bladders, detrusor overactivity without overt neurogenic cause (idiopathic), and diabetes. However, it is not always clear what the changes mean in terms of changes in detrusor function.

There is good evidence that outflow obstruction may change the cholinergic functions of the bladder. Thus detrusor denervation as a consequence of outflow obstruction has been demonstrated in several species including humans (240, 525, 592, 620). In detrusor from pigs with experimental outflow obstruction, Sibley (590) found that the response to intramural nerve stimulation was decreased, but that there was a supersensitivity of the detrusor to acetylcholine. Similar changes were found in bladders of obstructed patients with detrusor overactivity (260). Sibley (592) suggested the supersensitivity to be due to partial denervation of the bladder as a result of the obstruction and that one consequence of this may be detrusor overactivity. On the other hand, Yokoyama et al. (738) found that the responses to acetylcholine of detrusor strips from patients with detrusor overactivity were not significantly different from those without. The reasons for these conflicting results are unclear. Immunohistological investigations of the obstructed mouse bladder (also exhibiting detrusor overactivity) revealed that the nerve distribution patterns were markedly changed. In large parts of the detrusor, the smooth muscle bundles were completely devoid of accompanying vesicular acetylcholine transporter (VAChT)-immunoreactive varicose terminals. In other parts, the densities of nerve structures were clearly reduced (525). The obstructed human bladder often shows an increased (up to ~50%) atropine-resistant contractile component (56, 590, 596). This may be taken as indirect evidence of changes in the cholinergic functions of the bladder, since normally, the atropine-resistant component is almost negligible (18, 56, 636).

Kinder and Mundy (348) compared detrusor muscle from human normal, idiopathic overactive, and neurogenic overactive bladders. They found no significant differences in the degree of inhibition of electrically induced contractions produced by TTX or atropine in detrusor strips from any of these bladders and no significant differences in the concentration-response curves for acetylcholine. Bayliss et al. (56) found no atropine resistance in bladders with neurogenic detrusor overactivity but could demonstrate atropine-resistant contractions in those with idiopathic detrusor overactivity. In overactive bladders, without associated neurological disorders, a decreased number of muscarinic receptors was demonstrated (555), but its relation to overactivity remains unclear.

Conflicting results concerning changes in the cholinergic functions in neurogenic bladders have been reported. In patients with congenital neurogenic bladders (e.g., myelomeningocele) and detrusor dysfunction, Gup et al. (247) found no supersensitivity to muscarinic receptor stimulation and no changes in the binding properties of the muscarinic receptors. However, German et al. (225)

found that isolated detrusor strips from patients with neurogenic detrusor overactivity were supersensitive to both muscarinic receptor stimulation and KCl, but responded like normal controls to intramural nerve stimulation. The results were interpreted to suggest a state of postjunctional supersensitivity of the detrusor secondary to a partial parasympathetic denervation (225). An atropine-resistant component of the contraction of the human neurogenic bladder has been reported by some investigators (566), but not by others (56).

Patients with diabetes mellitus and voiding dysfunction show a variety of urodynamic abnormalities, including neurogenic detrusor overactivity and impaired detrusor contractility (322). In the detrusor smooth muscle of rats with diabetes, there were fewer spontaneous spike discharges, supersensitivity of postjunctional muscarinic receptors, reduced potency of the postjunctional Na^+-K^+ pump, and a decrease in the release of neurotransmitter, possibly due to the impairment of prejunctional activity (267). In bladders from diabetic animals, an increased density of muscarinic receptors accompanied by an enhanced muscarinic receptor-mediated phosphoinositide hydrolysis was found (376, 468). An upregulation of the M_2 receptor mRNA has been demonstrated in rats with streptozotocin-induced diabetes (657), and also that a supersensitivity of postjunctional muscarinic receptors may develop (267). However, it is unclear what these receptor changes mean for the functional bladder disorders seen in diabetic patients.

Interestingly, an aldose reductase inhibitor reduced the increase in muscarinic receptors and normalized the increased contractile response to acetylcholine seen in rats with diabetes induced by streptozotocin (320). Tong et al. (656) showed by Western immunoblotting using monoclonal antibodies that the amount of M_3 mAChR protein in diabetic bladders was significantly increased by ~70% compared with controls. Northern blotting demonstrated a 55% increase of M_3 mAChR mRNA in the diabetic bladder. These findings suggested an upregulation of M_3-mAChR biosynthesis in the diabetic urinary bladder. This phenomenon might explain the increased contractility-induced muscarinic stimulation of the detrusor muscle of diabetic animals (267).

B. Adrenergic Mechanisms

1. α-Adrenoceptors

The role of the sympathetic nervous system for human bladder function has been much discussed, partly because of the paucity of the adrenergic innervation of human detrusor muscle (239, 401). In most species, it is possible to evoke detrusor contraction with drugs stimulating α-adrenoceptors (ARs) on the detrusor muscle, preferentially with those acting on α_1-ARs, but high concentrations are needed (see Ref. 18). However, the possibility that released norepinephrine exerts an inhibitory effect on detrusor function by the adrenergic innervation through prejunctional inhibition of parasympathetic activation cannot be excluded. This may occur either at the level of intramural ganglia (607) and/or at the nerve terminal level (13, 458).

In isolated, normal human detrusor muscle, drugs selectively stimulating α-ARs produce a small and variable contractile effect (18). Goepel et al. (234), using ligand binding with [^{3}H]prazosin, were unable to detect any α_1-ARs in human detrusor. Walden et al. (699) reported a predominance of α_{1A}-AR mRNA, not only in the human trigone and bladder base, but also in the bladder dome. This contrasts with the findings of Malloy et al. (432), who found that only α_{1A}- and α_{1D}-mRNAs were expressed in the human bladder. The total α_1-AR expression was low (6.3 ± 1.0 fmol/mg total protein), and the relation between the different subtypes was α_{1D} 66% and α_{1A} 34% with no expression of α_{1B}.

Even if the functional importance of α-ARs in the normal human bladder can be questioned, there is a possibility that this may change in detrusor overactivity associated with, for example, outflow obstruction (532) and neurogenic damage (631), and that subtype expression may change. In rats with outflow obstruction, and a sixfold increase in bladder weight, the total α_1-AR density did not change, but the α_{1D}-ARs were upregulated (256). Thus, in control animals, 70% of α_1-AR mRNA were the α_{1A}-subtype, 5% were α_{1B}, and 25% were α_{1D}, whereas in obstructed animals bladder α_1-AR expression changed to 23% α_{1A}, 2% α_{1B}, and 75% α_{1D}. Changes in α_1-AR mRNA expression were of similar magnitude throughout the bladder dome, midbody, and base, and parallel changes were also evident at the protein level. A concurrent increase in voiding frequency versus before obstruction was observed. The authors concluded that their finding was potentially important since α_{1D}-ARs have a 10- to 100-fold higher affinity for the endogenous neurotransmitter norepinephrine than the α_{1A}- or α_{1B}-AR subtypes. As mentioned above, it was previously shown by the same group that the human bladder already has a predominance of α_{1D}-ARs (432).

It has been proposed that the balance between contraction-mediating α-ARs and relaxation-mediating β-ARs may be changed in bladder outflow obstruction so that there is a downregulation of the β-ARs (558, 664). Rohner et al. (558) found that in isolated dog detrusor muscle, which normally responds to norepinephrine with relaxation, contraction could be demonstrated in 7 of 12 animals after bladder outflow obstruction was established. This was suggested to be dependent on a decrease in β-AR function rather than to an increased α-AR function. Perlberg and Caine (532) found that norepinephrine caused contraction instead of the normal relaxant re-

sponse in bladder strips from 11 of 47 patients with bladder outflow obstruction. They proposed that there was a correlation between the response to stimulation on one hand and detrusor overactivity and irritative symptoms on the other. It has been observed that in patients with benign prostatic hyperplasia (BPH) treated with α-AR antagonists, detrusor overactivity may disappear during treatment (92). Taken together, these observations would suggest that there may be an increased α-AR function associated with the morphological changes occurring in bladder hypertrophy. On the other hand, Smith and Chapple (610) could not confirm the occurrence of an increased α-AR function in the overactive, obstructed bladder. Mattiasson et al. (459) found that the number of α-ARs in the detrusor, as determined by [^{3}H]dihydroergocryptine binding, was decreased in rats with bladder outflow obstruction. At the same time, α-AR-mediated contraction was impaired. Saito et al. (568), in contrast, found that in mildly obstructed rats, there was an increased detrusor response to phenylephrine, suggesting an enhanced α-AR function. It cannot be excluded that factors such as the degree and duration of obstruction have an important influence on α-AR-mediated responses in the detrusor.

Nomiya et al. (505) compared the expression level of α_1- and β-AR subtype mRNAs in normal and obstructed human bladders. In addition, they also performed an isometric contraction study to determine whether the α_1-AR-mediated contraction and β-AR-mediated relaxation of human detrusor muscle are altered by bladder outflow obstruction. Bladder outflow obstruction was confirmed by pressure-flow studies before the surgical removal of the bladder tissue specimens. In control bladders, α_{1A}-, α_{1B}-, α_{1D}-, β_1-, and β_2-AR mRNAs were expressed at very low levels, while the β_3-AR was the most highly expressed subtype. In obstructed bladders, the expressions of α_{1A}-, α_{1D}-, β_2-, and β_3-AR mRNAs were increased, whereas the expression of α_{1B}- and β_1-AR mRNAs were decreased. However, these changes were not significant. Phenylephrine at concentrations up to 10^{-5} M produced no response in the detrusor muscles from both control and obstructed bladder, and when responses were obtained (at high concentrations of phenylephrine), they were not increased in obstructed detrusor preparations. Isoprenaline and a β_3-AR selective agonist relaxed human detrusor muscle in a concentration-dependent manner. The relaxing effects of these agonists were the same in normal and obstructed detrusor muscle. Nomiya et al. (505) concluded that neither an upregulation of α_1-ARs nor a downregulation of β-ARs occurs in human obstructed bladder and that it was not likely that detrusor α_1-ARs are responsible for the overactivity observed in patients with bladder outflow obstruction.

Sundin and Dahlström (631) found that in parasympathetically decentralized cat bladder, there was a change in AR-mediated function with a shift from a β-AR dominated relaxant influence in the normal bladder to an α-AR dominated response after decentralization. However, other investigators were unable to confirm this finding (22, 431). Detrusor tissue from patients with detrusor overactivity (without neurological disorders) had an almost fourfold increase in the density of α-adrenoceptors compared with the density in normals (555). The importance of this finding for detrusor overactivity is, however, unclear.

2. β-Adrenoceptors

Norepinephrine is released by electrical stimulation of the adrenergic nerves in detrusor tissue (458). Because β-ARs have been shown to predominate over α-ARs, the response of the normal detrusor to norepinephrine is relaxation (532). It is theoretically possible, but has never been proven, that circulating epinephrine has a role in the activation of the β-ARs of the detrusor.

Early studies showed that in the detrusor of most species β_2-ARs predominate (18), but in, for example, guinea pig detrusor, which contains both β_1- and β_2-ARs, the relaxant effect was mediated mainly by β_1-ARs (396). In human detrusor, the β-ARs were shown to have functional characteristics typical of neither β_1- nor β_2-ARs, since they could be blocked by propranolol, but not by practolol (β_1) or butoxamine (β_2) (372, 496). With the use of different methods, including RT-PCR, direct sequencing of the PCR product, in situ hybridization, and isometric contractions in vitro, several investigators have been able to demonstrate that the human detrusor is able to express β_1-, β_2-, as well as β_3-ARs. Because selective β_3-AR agonists effectively relaxed human detrusor muscle (296, 297, 646, 732), this may mean that the most important β-AR for bladder relaxation is the β_3-AR, at least in humans. This can partly explain why the clinical effects of selective β_2-AR agonists in detrusor overactivity have been controversial and largely inconclusive (21). On the other hand, the β_2-AR agonist clenbuterol inhibited electrically evoked contractions in human "unstable," but not normal, bladder (290), which is in agreement with previous experiences in humans, suggesting that clenbuterol and also other β_2-AR agonists like terbutaline may inhibit detrusor overactivity (244, 404).

β_3-ARs have been demonstrated also in the detrusor of several animal species (642–646, 734–736). However, various studies have concluded that the relaxant response of, e.g., rat bladder to isoprenaline and other nonselective β-AR agonists, is mediated by both β_2- and β_3-ARs (299, 414, 519, 642, 644, 721, 736). Furthermore, mRNAs for β_1-, β_2-, and β_3-ARs have been detected in rat bladder using RT-PCR (209, 579). β_3-AR agonists have shown relaxant effects in vitro and in animal models of detrusor overactivity (296, 297, 318, 476, 645, 646, 648–650, 721, 734).

However, no proof of concept studies seem to have been performed humans, showing that this is an effective principle to treat detrusor overactivity.

As mentioned previously, it may be speculated that in detrusor overactivity there is a lack of the inhibitory β-AR-mediated norepinephrine response. However, detrusor muscle from patients with detrusor overactivity was reported to show a similar degree of inhibition in response to isoprenaline as normal detrusor (161), even if the inhibitory effect of isoprenaline on the response to electrical stimulation was less in overactive muscle. No differences in the density of β-ARs were revealed by receptor binding studies between normal and overactive human detrusors (555).

β-AR agonists are considered to stimulate adenylyl cyclase to increase cAMP. In turn, cAMP activates PKA to mediate their biological effects. Nakahira et al. (492) showed in guinea pigs that isoprenaline prevented spontaneous action potential discharges and associated calcium transients through the activation of PKA. The isoprenaline-induced inhibition of intracellular Ca^{2+} largely depended on the prevention of spontaneous action potentials, since the contribution of the intracellular calcium store was small. Isoprenaline hyperpolarized the cell membrane, probably by stimulating sodium pump activity. Nakahira et al. (492) also found no effect of different K^+ channel blockers on the hyperpolarization and concluded that activation of K^+ channels was not involved in this effect. This is in contrast to the findings of Kobayashi et al. (359), who reported that the isoprenaline-induced relaxation of guinea pig bladder smooth muscle was mainly mediated by facilitation of BK_{Ca} channels subsequent to the activation of the cAMP/PKA pathway. Furthermore, Hudman et al. (289) suggested that K_{ATP} channel opening and the subsequent hyperpolarization of cell membranes in response to β_2-adrenoceptor activation is mediated by raised cAMP levels and activation of PKA.

There are many pieces of evidence from studies in various animals suggesting that the sympathetic nervous system contributes to the urine storage function by inhibiting the reflex activation of the detrusor muscle during bladder filling (see Ref. 18). However, the role of β-AR-mediated detrusor relaxation in humans has been questioned (18). One argument is that β-AR blockade seems to have no effect on normal human detrusor function; another is that individuals lacking dopamine β-hydroxylase, which is necessary for norepinephrine synthesis, seem to have normal bladder function (221). Thus the functional importance of β-ARs for normal detrusor function in humans remains to be settled.

C. NANC Mechanisms

It has been known for a long time that in most mammalian species, part of the neuronally induced bladder contraction is resistant to atropine (see Ref. 18). In the rat bladder, where two different components of the contractile response to electrical stimulation can be detected, both in vitro (392, 430, 526) and in vivo (294, 407), the fast response is elicited by a NANC transmitter.

The proportion of NANC-mediated response to the total contraction seems to vary with species and the frequency of stimulation. Thus, in bladder strips from rats and guinea pigs, atropine had little effect on the response to single nerve stimuli, but at 20 Hz, it inhibited ~25% of the response. Corresponding figures for rabbit and pig were 40 and 75%, respectively (74). In mice, atropine reduced the response to electrical stimulation only by 30% of maximum (729). In female mice lacking both M_3 and M_2 receptors, the atropine-resistant response to electrical nerve stimulation was increased compared with controls (116 vs. 84% of K^+-induced contraction), whereas in males, it was decreased (40 vs. 102% of K^+-induced contraction) (453).

The role of a NANC mechanism in the contractile activation of the human bladder is still disputed (18, 147). Cowan and Daniel (132) found that acetylcholine was responsible for ~50% of the electrically induced contraction in strips of the normal human detrusor. In contrast, Sjögren et al. (596), who investigated morphologically normal detrusor samples from patients undergoing bladder surgery for various reasons, found that atropine caused >95% inhibition of electrically evoked contractions. Sibley (590) compared the effects of atropine on contractions induced by electrical field stimulation in isolated detrusor preparations from rabbit, pig, and humans. He found that in human preparations, obtained from patients undergoing lower urinary tract surgery for different disorders, or donor nephrectomy, atropine abolished nerve-mediated contractions. He concluded that nerve-mediated contractile activity in human detrusor is exclusively cholinergic. This was supported by the results of Kinder and Mundy (347), who found that atropine caused an almost total inhibition of the electrically induced contraction in human detrusor tissue taken from patients with urodynamically normal bladders. Luheshi and Zar (416) investigated whether the reported full atropine sensitivity of the human detrusor was due to a genuine absence of a noncholinergic element in its motor transmission, or was dependent on the experimental protocols, which in most investigations involve prolonged electrical stimulation. They used an experimental protocol where field stimulation was limited to the minimum required to elicit consistent and reproducible contractions, and found that part of the electrically induced response (~30%) was resistant to atropine. With a more conventional stimulation protocol involving long trains of pulses, however, the responses were enhanced by physostigmine and fully blocked by atropine. Atropine-resistant, TTX-sensitive contractions evoked by electrical stimulation in normal

human detrusor tissue also have been reported by other investigators (56, 287, 564). However, the contribution of NANC neurotransmission to bladder excitation in humans, and also in pigs, seems to be small. Tagliani et al. (636) concluded that the atropine-resistant component may reflect direct smooth muscle excitation, since the human detrusor has a very short chronaxie.

These apparently conflicting data may partly be explained by differences in the tissue materials investigated and by varying experimental approaches. Most probably, normal human detrusor muscle exhibits little "atropine resistance." This does not exclude that atropine resistance may exist in morphologically and/or functionally changed bladders. Sjögren et al. (596) found that in detrusor strips from male patients with a diagnosis of detrusor overactivity, and in particular from patients with bladder hypertrophy, an atropine-resistant component of up to 50% of the electrically induced contraction could be demonstrated. This was confirmed by Smith and Chapple (610), who compared the responses to electrical stimulation of detrusor strips from normal individuals and from patients with bladder outflow obstruction, with or without detrusor overactivity. They found a NANC response only in strips from patients with detrusor overactivity (15/21), amounting to 25% of the original response. Nergårdh and Kinn (497) found a varying degree of atropine resistance (0–65%) in isolated detrusor preparations from male patients, most of them having outflow obstruction. Sibley (590) verified the occurrence of atropine resistance in hypertrophic bladder muscle, but also showed that the atropine resistant response was resistant to TTX, suggesting that it was not nerve mediated but was caused by direct muscle stimulation. This finding was not confirmed by Smith and Chapple (610), who showed that the NANC response from overactive, obstructed bladders was eliminated by TTX. Yoshida et al. (739) found a significant positive correlation between age and the NANC response and a significant negative correlation between age and the cholinergic neurotransmissions in human isolated detrusor preparations. The NANC component of the nerve-induced response may be responsible for up to 40–50% of the total bladder contraction in different conditions associated with detrusor overactivity (56, 347, 515, 522, 590, 596).

Taken together, these results suggest that there is a NANC component contributing to motor transmission in the isolated human detrusor and that no obvious qualitative difference exists between humans and other mammalian species. In normal detrusor, this component of contraction seems to be small. However, the importance of the NANC component for detrusor contraction in vivo, normally, and in different micturition disorders, remains to be established.

1. ATP

Stimulation of nerves in the guinea pig bladder evokes contractions that, at least partially, can be mimicked by exogenous ATP, and studies on the NANC component of bladder contraction in this species suggested that the excitatory transmitter is ATP (89). Excitatory purinergic responses have been reported in bladders from several other species (see Refs. 18, 89). ATP acts on two families of purinergic receptors: an ion channel family (P2X) and a G protein-coupled receptor family (P2Y). Seven P2X subtypes and eight P2Y subtypes have been identified (336, 509, 554). In, e.g., rabbit, cat, and rat, various studies suggested that multiple purinergic excitatory receptors are present in the bladder (89). The detrusor response to ATP seems to be mediated by activation of a ligand-gated cation channel (P2X receptor) that promotes the influx of extracellular Ca^{2+}, whereas uridine triphosphate and adenosine 5′-*O*-(2-thiodiphosphate) would be expected to act through G protein-coupled receptors ($P2Y_2$ or $P2Y_4$) to induce smooth muscle contractions via a phospholipase C/IP_3 signaling pathway (90) and release of intracellular Ca^{2+}. In the rabbit bladder, the response to ATP is normally biphasic, but after desensitization with α,β-methylene-ATP, the response is monophasic, suggesting two different types of receptors (115). Interestingly, Wibberley et al. (715) found that contractions induced by α,β-methylene-ATP were significantly inhibited by the Rho-kinase inhibitor Y-27632, but the mechanism behind this effect was not determined.

Ligand receptor binding studies (rabbit and rat) revealed high-affinity binding of [^{3}H]ATP and α,β-[^{3}H]methylene-ATP to bladder membranes (90). Displacement experiments with various agonists and antagonists indicated the presence of a P2X receptor (62, 63). Immunohistochemical experiments with specific antibodies for different P2X receptors showed that $P2X_1$ receptors are the dominant subtype in membranes of rat and mouse detrusor muscle (380, 687). In adult human bladders, O'Reilly et al. (514) found that $P2X_1$ was by far the predominant purinergic receptor at the mRNA level. The remaining purinergic receptors were consistently present in the order $P2X_1 >> P2X_4 > P2X_7 >> P2X_5 > P2X_2 >> P2X_3 = P2X_6 = 0$. In fetal bladders the expression of $P2X_1$ transcripts was much lower than in adult bladders, and $P2X_4$ and $P2X_7$ were also present. The rank order of the P2X transcript level was $P2X_1 = P2X_4 > P2X_7 >> P2X_5 >> P2X_2 >> P2X_3 = P2X_6 = 0$. With increasing gestation the P2X receptor transcript level (expression) shifted from the dome to the body of the bladder. A spliced variant of the $P2X_1$ receptor has been demonstrated in the human detrusor (257), which most probably can express multiple isoforms of this receptor.

Thus there is good evidence that the transmitter responsible for the NANC component of detrusor contrac-

tion is ATP (89), acting on P2X receptors, and as mentioned above, the receptor subtype, which is predominating and mediating contraction in various species, seems to be the $P2X_1$ subtype. Changes in P2X receptor subtypes in bladders from patients with idiopathic detrusor overactivity have been reported (473, 515). O'Reilly et al. (515) were unable to detect a purinergic component of nerve-mediated contractions in control (normal) bladder preparations but found a significant component in overactive bladder specimens, where the purinergic component was ~50%. They concluded that this abnormal purinergic transmission in the bladder might explain symptoms in these patients. ATP was a more potent contractile agonist in bladder preparations from patients with overactive and obstructive bladders than in specimens from normal bladders (263, 291), a finding suggested to be contributing to detrusor overactivity. Wu et al. (723) found that the generation of purinergic contractions in overactive detrusors was not due to altered sensitivities of the detrusor myocyte to ATP or cholinergic agonists. Instead, Harvey et al. (263) reported that the greater potency of ATP for generating contractions in detrusor from overactive bladders may be due to reduced extracellular hydrolysis, allowing purines greater access to detrusor smooth muscle, and that this finding may explain atropine-resistant purine-based contractions in detrusor from overactive bladders.

O'Reilly et al. (514) confirmed that the $P2X_1$ receptor was the predominant purinoceptor subtype in the human male bladder. They also found that the amount of $P2X_1$ receptor per smooth muscle cell was greater in the obstructed than in control bladders. This suggests an increase in purinergic function in the overactive bladder arising from bladder outflow obstruction. Such a view was supported by experiments in rabbits, showing that the purinergic component of nerve-mediated detrusor contraction is increased and the cholinergic component is decreased in early stages of bladder obstruction (97). In contrast, in the obstructed rat bladder, Sjuve et al. (601) found that the force of the contractions induced by the purinoceptor agonists, ATP and α,β-methylene-ATP, was significantly lower in the hypertrophied bladder compared with the controls. The reasons for this discrepancy are unclear. Whether or not abnormalities in the purinergic transmission in the bladder can explain overactive bladder symptoms, in idiopathic detrusor overactivity in women and in men with bladder outflow obstruction, has not been established. If this is the case, abnormal purinergic activation of the detrusor may explain why antimuscarinic treatment fails in a number of patients.

2. Nitric oxide

L-Arginine-derived nitric oxide (NO) seems to be responsible for the main part of the inhibitory NANC responses in the lower urinary tract (18, 26, 484). Both constitutive NO synthase (nNOS, eNOS) and inducible NO synthase (iNOS) can be demonstrated in lower urinary tract smooth muscle from animals and humans (154, 169, 170, 187, 315, 382, 384, 450, 451, 513). There is so far no evidence that nNOS is produced by detrusor smooth muscle cells, and in unstimulated detrusor cells, iNOS was not detected. However, the cells expressed the enzyme when exposed to lipopolysaccharide, or cytokines known to be produced during urinary tract infections (315, 513, 706). Bladder outflow obstruction can cause an increase in iNOS activity and a decrease in nNOS activity (187, 314, 384). Thus expression of iNOS was enhanced, both at the cDNA and protein levels, 1 and 3 wk after experimental outflow obstruction in mice, suggesting that iNOS-derived NO is involved in the early response to bladder outlet obstruction (382). It was speculated that the enhanced iNOS expression was a response to overcome the effects of obstruction-induced ischemia. Felsen et al. (187) presented evidence that iNOS derived NO, forming reactive nitrogen species, promoted the spontaneous bladder contractions and fibrosis induced by outlet obstruction. They also suggested that iNOS inhibitors may have a therapeutic potential.

The functional role of NO in the detrusor has not been established. Klarskov (356) reported a NANC-mediated relaxation of pig and human detrusor muscle preparations in response to electrical stimulation. In pig detrusor, this relaxation was preceded by a contraction and was possible to block by TTX. In the human detrusor, the relaxation was seen only occasionally, and was short-lasting and fading. Its TTX sensitivity was apparently not tested. In small biopsy preparations of the human detrusor, James et al. (307) found that electrical stimulation evoked relaxations sensitive to N^G-nitro-L-arginine, but insensitive to TTX. They suggested that NO might be generated from the detrusor muscle and an important factor for bladder relaxation during the filling phase. Elliott and Castleden (176) were unable to demonstrate a nerve-mediated relaxation in human detrusor muscle. In pig detrusor contracted by K^+ (35 mM) after pretreatment with atropine and α,β-methylene-ATP, no response or small contractions were found. If, instead, contraction was induced by endothelin-1 (ET-1) in a concentration inducing tension near that evoked by high K^+ (124 mM), a small relaxation was seen in some preparations, which was sensitive to N^G-nitro-L-arginine, but partly insensitive to TTX (534). Electrical stimulation of the precontracted rat detrusor in no case produced relaxation, but further contraction (536).

The normal bladder responds to filling at a physiological rate with relaxtion and can accommodate large volumes of urine, with a minimal increase in intravesicular pressure (130). There have been many suggestions regarding the underlying mechanism (19). The phenomenon has been attributed not only to the physical proper-

ties of the bladder, but also to the existence of an inhibitory neural mechanism operative during filling/storage. Inhibition of parasympathetic nervous activity (357), or an increase in sympathetic nervous activity (146), has been suggested, but also involvement of NO, generated within the bladder (653). If NO has an important role in detrusor relaxation, it may be expected that the detrusor muscle has a high sensitivity to agents acting by increasing the intracellular concentrations of cGMP. In the pig detrusor, the NO donor SIN-1 and NO relaxed carbachol, and ET-1 contracted preparations by ~60%. However, isoprenaline was ~1,000 times more potent than SIN-1 and NO and caused complete relaxation. Nitroprusside, SIN-1, and NO were only moderately effective in relaxing isolated rat, pig, and rabbit detrusor muscle, compared with their effects on the urethral muscle (533, 534). These results agree well with those of Morita et al. (477), who found that in rabbits, cGMP is mainly related to urethral relaxation and cAMP to urinary bladder relaxation. They also agree with the findings of Masuda and co-workers (450, 451) demonstrating that in the detrusor, in contrast to the urethra, NOS and soluble guanylyl activities were mainly detected in the mucosa, and not in the smooth muscle. In the human bladder, Moon (472) found that both NO donors and dibutyryl cGMP evoked contraction, relaxation, or both. She suggested that there probably is a NO/cGMP signaling pathway in the human detrusor. It may be that this involves the interstitial cells expressing cGMP, which were found throughout the human bladder body (607).

It is well established that protein kinase G (PKG, cGK) is essential for NO signaling. PKG has several effects in the cell leading to muscle relaxation, including desensitization of the contractile machinery to Ca^{2+} (cf. sect. IV*A*) and activation of BK_{Ca} channels (284, 577). The importance of cGK I for lower urinary tract function in mice lacking the gene for cGK I (cGK I −/−) and in litter-matched wild-type mice (cGK I +/+), studied in vitro and in vivo, revealed that cGK I deficiency did not result in any changes in bladder gross morphology or weight. Bladder strips from cGK I −/− mice responded like controls to electrical field stimulation and to carbachol, but not to 8-bromo-cGMP (537). However, cGK I −/− mice exhibited detrusor overactivity, which was attributed to effects on the outflow region (537).

Taken together, available results suggest that it is unlikely that NO has a role as a neurotransmitter causing direct relaxation of the detrusor smooth muscle. This does not exclude that NO may modulate the effects of other transmitters, or that it has a role in afferent neurotransmission. For example, NO was found to have a growth inhibitory effect in bladder smooth muscle cells, suggesting that changes in NOS activity may influence the progress of bladder hypertrophy (187, 314, 384).

3. Neuropeptides

The functional roles of the many neuropeptides that have been demonstrated to be synthetized, stored, and released in the human lower urinary tract (422, 424) have not been established. Several aspects of neuropeptides in the lower urinary tract have been reviewed previously (18). Studies on isolated human detrusor have suggested that, e.g., vasoactive intestinal polypeptide, ET-1, tachykinins, and angiotensins, may be involved in contraction and relaxation as well as in adaptive growth. These neuropeptides will be discussed in this review. Whether or not neuropeptides are involved in various forms of detrusor overactivity is at present unclear.

A) VASOACTIVE INTESTINAL POLYPEPTIDE. Vasoactive intestinal polypeptide (VIP) is known to bind to two types of receptor, $VPAC_1$ and VPC_2, in several types of smooth muscle (258), including the human urinary bladder (556). Both receptor subtypes are G protein coupled (G_s). The effects of VIP on isolated urinary detrusor vary from species to species. In isolated detrusor muscle from humans, the peptide inhibited spontaneous contractile activity, but was found to have little effect on contractions induced by muscarinic receptor stimulation or by electrical stimulation of nerves (349, 597). Uckert et al. (668), on the other hand, demonstrated a concentration-dependent relaxation of carbachol-induced contractions in human detrusor strips. This effect was paralleled by a three- to fourfold elevation in tissue cGMP and a twofold rise in cAMP, suggesting stimulation of both guanylyl cyclase and adenylyl cyclase. In detrusor strips from rat and guinea pig, no effect, or contraction, was demonstrated, whereas relaxation and inhibition of spontaneous contractile activity was found in preparations of the the rabbit detrusor (28, 177, 190, 295, 393, 597).

If the low-amplitude myogenic contractile activity demonstrated in human as well as animal bladders is of primary importance for the genesis of reflex bladder contraction, direct inhibitory effects of VIP on bladder smooth muscle may be physiologically important. The findings that VIP levels were markedly reduced in patients suffering from idiopathic detrusor overactivity (105, 245) or neurogenic detrusor overactivity (349) were interpreted to suggest that VIP (or rather lack of it) may be involved in some forms of detrusor overactivity. On the other hand, in rats with infravesicular outflow obstruction, bladder hypertrophy, and detrusor overactivity, the concentrations of VIP in the middle and lower parts of obstructed bladders were higher than in controls (28). Neither in the hypertrophic nor in the normal isolated rat bladder did VIP have relaxant or contractant effects, and the peptide did not influence contractions induced by electrical stimulation (28, 295). This does not support the view that lack of VIP is associated with detrusor overactivity, at least not in the rat.

B) ENDOTHELINS. The presence of endothelins (ETs) in animal and human detrusor has been well established. Garcia-Pascual et al. (219) found ^{125}I-ET-1 binding sites mainly in the outer longitudinal muscle layer, in vessels and in the submucosa of the rabbit bladder. The highest density of binding sites appeared to be in vessels and the outer muscle layer. Saenz de Tejada et al. (565) demonstrated in both human and rabbit bladder, ET-like immunoreactivity in the transitional epithelium, serosal mesothelium, vascular endothelium, smooth muscles of the detrusor and vessels, and in fibroblasts. This cellular distribution was confirmed in in situ hybridization experiments.

ET-1 is known to induce contraction in animal as well as human detrusor muscle (see Ref. 18). Maggi et al. (426, 427) demonstrated that ET-1, as well as ET-3, produced slowly developing, concentration-dependent contractions of the human detrusor preparations. ET-3 was less potent than ET-1. Garcia-Pascual et al. (219) showed similar effects in rabbit detrusor preparations. There was a marked tachyphylaxis to the effects of the peptide. The ET-1-induced contractions were not significantly affected by scopolamine or indomethacin, but could be abolished by incubation in a Ca^{2+}-free solution, and nifedipine had a marked inhibitory action. On the other hand, in human detrusor, the ET-1-induced contractions were resistant to nifedipine (427), illustrating species variation in the activation mechanisms. In rabbit bladder, Traish et al. (661) found that ET-1, ET-2, and ET-3 all caused concentration-dependent contractions. The threshold concentrations of ET-3 to initiate contraction were higher than for ET-1 and ET-2. Traish et al. (661) also characterized the ET receptor subtypes in the rabbit bladder using radioligand binding and suggested that at least two subtypes exist in rabbit bladder tissue, ET-1 and ET-2 binding to one subpopulation (ET_A) and ET-3 to the other (ET_B). The main role of ET_A receptors in the contractile effects of ETs in the detrusor has been confirmed by several other investigators in animal as well as human bladders (96, 155, 375, 512). In human detrusor, Okamoto-Koizumi et al. (512), using isometric contraction experiments and RT-PCR, demonstrated that the ET-1-induced contractions were mediated mainly by the ET_A receptor and not by the ET_B receptor. RT-PCR revealed positive amplification of the ET_A, but not ET_B, receptor mRNA fragments (512).

The contractile effects of the ETs in the detrusor seem to involve both activation of L-type Ca^{2+} channels and activation of phospholipase C (219, 220, 427, 535). Thus, in the pig detrusor, contractile effects were associated with an increase in IP_3 concentrations and were blocked by the PKC inhibitor H-7 and by nifedipine (535).

The functional role of ETs in the detrusor has not been established. The slow onset of the contractile effects seems to preclude direct participation in bladder emptying. However, Donoso et al. (155) found in the rat bladder that ET-1 potentiated the contractions evoked by both transmural nerve stimulation and applications of ATP at peptide concentrations 10-fold below those needed to produce an increase in bladder tone. This suggests a modulatory effect on detrusor neurotransmission. The expression of ET_A receptors has been observed to increase in bladder smooth muscle cells isolated from a rabbit model of bladder outflow obstruction (337). Because a mitogenic effect of ET-1 is well established, this suggests that ET receptors can be involved in detrusor hyperplasia and hypertrophy associated with bladder outflow obstruction.

C) TACHYKININS. Tachykinins, including substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), are present in capsaicin-sensitive, primary afferent nerves of the urinary bladder in rat and other mammalian species, including humans (379, 424, 425). Even if it seems generally accepted that tachykinins have mainly afferent functions, peripheral release of these peptides may have important effects on detrusor function by inducing "neurogenic inflammation" (379, 425).

The tachykinins act on NK1, NK2, and NK3 receptors, and SP, NKA, and NKB possess the highest affinity for NK1, NK2, and NK3 receptors, respectively. All receptor subtypes have been identified in urinary bladders of several mammals, both in vitro and in vivo (379, 425). In the rat detrusor, NK1, NK2, and NK3 receptors have been demonstrated, as evidenced by radioligand binding, autoradiographic, and functional experiments, whereas in hamster, mouse, dog, and human detrusor, NK2 receptors predominate (379).

In the human detrusor, the presence of tachykinins, their receptors, and their contractile effects are well documented (87, 150, 179, 231, 429, 523, 608, 712, 746). Their potencies were shown to be NKA $>$ NKB $>>$ SP (150, 429). Together, the results support the view that the tachykinin receptor mediating contraction in the human bladder is of the NK2 type.

The NK2 receptor-mediated contraction in the human detrusor is largely dependent on the activation of L-type Ca^{2+} channels, and sensitive to nifedipine (429). However, a role of intracellular Ca^{2+} cannot be excluded, since NK2 stimulation also activates phospholipase C (448, 630). Prostanoids generated following NK2 receptor activation may amplify the direct contractile effect of NK2 receptor stimulation (662). Interestingly, Wibberley et al. (715) found that NKA-induced contractions of the rat detrusor could be attenuated by the Rho-kinase inhibitor Y-27632, suggesting involvement of the Rho-kinase pathway.

D) ANGIOTENSINS. An autocrine/paracrine renin-angiotensin system has been identified in the urinary bladder (704), and angiotensin II (ANG II) formation was demonstrated in human isolated detrusor smooth muscle (24, 403, 698). ANG II receptors have been demonstrated by

different methods in animal as well as human detrusor (16, 370). ANG II was reported to contract the urinary bladder of several species, but with a wide range of relative potencies (16, 178). Several investigators (24, 179, 403, 567, 698), but not all (370), have shown that in human detrusor muscle, ANG II was a potent and effective contractile agent. In the dog bladder, the responses to both ANG II and ANG I were minor or lacking (622), illustrating the wide variation in response to the peptide between species.

The responses in human detrusor were antagonized by the AT_1 receptor antagonist losartan, but not by the AT_2 receptor antagonist PD-123319, indicating interaction with the AT_1 receptor (370). Also in the rat bladder AT_1 receptors mediated the contractile effect of ANG II (647). ANG I caused concentration-dependent contractions in the human detrusor, which, like those evoked by ANG II, could be blocked by saralasin. This suggests that the actions of both ANG I and ANG II were mediated through stimulation of ANG II receptors (24). The contractile effect of ANG II was very sensitive to removal of extracellular calcium, but less so to calcium antagonists, suggesting that calcium influx may occur through pathways beside L-type Ca^{2+} channels (24, 567).

The effects of ANG I could not be blocked by the angiotensin converting enzyme inhibitors captopril and enalaprilate (24). Further studies revealed that a serine protease was responsible for ANG II formation in the human bladder in vitro, probably human chymase or an enzyme similar to human chymase (403, 698).

The functional importance of ANG II in the detrusor has not been established. The delayed onset of action of the contractile effect of exogenous ANG II, and the fact that saralasin was not able to block completely the atropine-resistant component of electrically induced contractions, made Anderson et al. (16) suggest that if ANG II is involved in neurotransmission, it may be as a neuromodulator. Based on experiments in rabbits (113, 114), Cheng et al. (112) suggested that *1*) outlet obstruction of the bladder can cause increased cell stretch/strain, which in turn induces the local production of ANG II. ANG II may also influence cell stretch/strain via its direct effects on bladder tone. *2*) ANG II then acts as a trophic factor in the bladder wall to cause smooth muscle cell hypertrophy/hyperplasia and increased collagen production via an autocrine and/or paracrine pathway. *3*) The cellular effect(s) of ANG II may be mediated by secondary growth factors such as bFGF and transforming growth factor-β. Stretch-stimulated growth of rat bladder smooth muscle cells was shown to involve the ANG II receptor system (501). On the other hand, inhibition of ACE or blockade of ANG II receptors had no effect on the development of bladder hypertrophy in rats (524, 538).

4. Prostanoids

Prostanoids (prostaglandins and thromboxanes) are synthesized by cyclooxygenase (COX) in the bladder (18, 338, 423). This enzyme exists in two isoforms, one constitutive (COX-1) and one inducible (COX-2). It has been suggested that in the bladder, the constitutive form is responsible for the normal physiological biosynthesis, whereas the inducible COX-2 is activated during inflammation (662). Prostanoids are generated locally in both detrusor muscle and mucosa (83, 156, 310, 338), and its synthesis is initiated by various physiological stimuli such as stretch of the detrusor muscle (228), but also by injuries of the vesical mucosa, nerve stimulation, and by agents such as ATP and mediators of inflammation, e.g., bradykinin and the chemotactic peptide formyl-methionyl-leucyl-phenylalanine (see Refs. 18, 338, 423).

There seem to be species variations in the spectrum of prostanoids and the relative amounts synthesized and released by the urinary bladder. Biopsies from the human bladder were shown to release prostaglandins (PG) and thromboxane A_2 (TxA_2) in the following quantitative order: $PGI_2 > PGE_2 > PGF_{2\alpha} > TxA_2$ (310).

The actions of prostanoids are mediated by specific receptors on cell membranes. The receptors include the DP, EP, FP, IP, and TP receptors that preferentially respond to PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and TxA_2, respectively. Furthermore, EP is subdivided into four subtypes: EP_1, EP_2, EP_3, and EP_4 (81, 495). The signaling pathways vary. For example, TP receptors are known to signal via the G_q G protein, activating Ca^{2+}/diacylglycerol pathways, but also other G proteins may be involved. EP_1 receptors signal via IP_3 generation and increased cell Ca^{2+}, activation of EP_2 and EP_4 leads to an increase in cAMP, and EP_3 activation seems to inhibit cAMP generation via a pertussis toxin-sensitive G_i-coupled mechanism and may also signal via the small G protein Rho (81).

Several investigators have shown that $PGF_{2\alpha}$, PGE_1, and PGE_2 contract isolated human, as well as animal, detrusor muscle (18), which has led to the suggestion that they contribute to the maintenance of detrusor tone. Prostanoids may affect the excitation-contraction coupling in the bladder smooth muscle in two ways, directly by effects on the smooth muscle, and/or indirectly via effects on neurotransmission. The membrane potential of guinea pig and rabbit smooth muscle cells was unchanged by low concentrations of PGE_2 (up to 10^{-6} M), but at higher concentrations the cells depolarized and the frequency of spontaneous action potentials increased. It was concluded that prostanoids are not normally released by the nerves to the guinea pig urinary bladder. They are able to facilitate excitation-contraction coupling, possibly by mobilizing Ca^{2+} (136). The contractile response of detrusor muscle to prostanoids is slow, and it is unlikely that these agents are directly involved in the evacuation of the blad-

der by exerting direct effects on the detrusor smooth muscle (27).

The prostanoid receptor most important for detrusor function has not been established. Mice lacking EP_1 receptors had normal cystometry, but did not react to intravesical PGE_2 instillation, which caused detrusor overactivity in wild-type controls (576a). Probably, prostanoids do not act as true effector messengers along the efferent arm of the micturition reflex, but rather as neuromodulators of the efferent and afferent neurotransmission; they may also have other functions in the bladder (18, 338, 423). It has been demonstrated that the expression of COX-2 is increased during bladder obstruction (529) and that this can be a response to mechanical stress (528). Obstruction of EP_1 receptor KO mice did not prevent the resulting increase in bladder weight, but prevented the increase in spontaneous contractile activity (nonvoiding contractions) seen in the wild-type controls (576a).

VII. SUMMARY AND FUTURE PERSPECTIVES

The detrusor is a comparatively fast, "phasic," smooth muscle with expression of fast types of smooth muscle myosin isoforms. The cytoskeleton and contractile system of the detrusor muscle have a large potential for adaptation in response to different pathophysiological conditions. The detrusor smooth muscle cell generates spontaneous action potentials associated with increases in intracellular Ca^{2+} and myogenic tone. Intercellular coupling via gap junctions may contribute to spread of excitation. The cells seem to work in functional units, which need nervous synchronization to generate a voiding contraction of the whole bladder. The variations in $[Ca^{2+}]_i$ activate the contractile machinery via myosin light-chain phosphorylation. This process is modulated by a cellular signaling network that can alter the relations between Ca^{2+}, myosin phosphorylation, and contraction.

The detrusor muscle contains receptors for numerous transmitters/modulators, released from nerves or generated locally. The most important receptors for activation of contraction are muscarinic (M_3) and purinergic receptors ($P2X_1$). The contribution of these receptors to contraction may differ between species. In the normal human detrusor, the muscarinic component predominates; however, this contribution may change in different pathophysiological conditions. The main relaxant pathway is via the adenylyl cyclase/cAMP pathway, which is activated by adrenergic β_3-receptors, although other relaxant pathways also may contribute.

Although our understanding regarding bladder function has increased during recent years, the detrusor muscle still provides a considerable challenge for future basic, clinical, and translational research. Patients with lower urinary tract symptoms constitute a large and growing population, and increased knowledge regarding the pathobiology and possible therapeutic targets is urgently needed. One such area is the adaptive changes in the detrusor in response to outflow obstruction. Several questions regarding different growth factors, the local angiotensin and endothelin systems, and the cellular signaling pathways, which initiate the cellular hypertrophy in bladder outflow obstruction, need to be addressed. Changes in the detrusor muscle caused by diabetes, neurological disorders (e.g., Parkinson's disease), and old age are important areas for future investigation.

Another line of research is the creation of new detrusor tissue, "tissue engineering," and the morphological and function characterization of such tissue at the cellular tissue and organ level (45, 369). This field has not been covered in the present review but is rapidly developing. Attempts to use gene therapy for modification of detrusor function have already been initiated (124) and may open new fields for therapy of different disorders of the lower urinary tract.

From a cell biology or a basic physiology perspective, the detrusor muscle exhibits interesting properties. It has a remarkable ability to change in length, corresponding to the ability of the bladder to store urine at low intravesical pressures. The mechanisms involved in this mechanical adaptation remain unknown. The structure and kinetics of the contractile system can be modulated in vivo by processes that are not understood at present. Although the smooth muscle cells constitute the majority of cells in the bladder wall, small numbers of nonsmooth muscle cells may have important functions in coordinating contractions, as volume sensors, in release of local factors and possibly as smooth muscle precursors during growth. In future research, the interaction between the detrusor smooth muscle cells and other cell types within or close to the detrusor will be important to characterize in the normal urinary bladder as well as in bladders with adaptive changes or exposed to injury.

Thus, despite extensive research for more than a decade, the detrusor smooth muscle remains an interesting and fruitful field for further research.

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