Possible role of bioactive peptides in the regulation of human detrusor smooth muscle – functional effects in vitro and immunohistochemical presence

Abstract Results from basic research implicate a role for bioactive peptides in controlling the mammalian lower urinary tract. Although various peptides are assumed to be involved in the potentiation or inhibition of cholinergic or purinergic activity in the urinary bladder, there is still much controversy regarding the mode of action and functional significance of such peptides in detrusor smooth muscle. Thus, we evaluated the functional effects of atrial natriuretic peptide (ANP), calcitonin gene related peptide (CGRP), endothelin 1 (ET-1), substance P (SP) and vasoactive intestinal polypeptide (VIP) on isolated strip preparations of human detrusor smooth muscle and determined the presence of those peptides in the human detrusor by means of immunohistochemistry. The effects of peptides on isometric tension of isolated detrusor strip preparations and on tissue levels of cyclic nucleotides cAMP and cGMP were compared to those of adenylyl cyclase activator forskolin (F), nitric oxide donor Na+-nitroprusside (SNP) and non-specific phosphodiesterase (PDE) inhibitor papaverine (P). The effects of the compounds on isometric tension of isolated human detrusor smooth muscle were examined using the organ bath technique. To determine time- and dose-dependent effects on cyclic nucleotide levels, bladder strips were exposed to increasing doses of F, SNP, P, ANP, CGRP and VIP, then rapidly frozen in liquid nitrogen and homogenised in the frozen state. cAMP and cGMP were extracted and assayed using specific radioimmunoassays. The presence of peptides was investigated by light microscopy using the Avidin-Biotin-Complex (ABC) method. F, P and VIP most effectively reversed the carbachol-induced tension of isolated human detrusor strips. Relaxing effects of ANP, CGRP and SNP were negligible. In contrast, ET-1 and SP elicited dose-dependent contractions of the tissue. The relaxing effects of F, P and VIP were accompanied by an increase in cAMP and cGMP levels, respectively. Light microscopy revealed positive immunostaining for CGRP, ET 1, VIP and SP in sections of the detrusor muscle coat. Our results suggest a possible importance of ET 1, SP and VIP in regulating detrusor smooth muscle contraction and relaxation. Even if a peptide is not synthesised, stored or released in a smooth muscle tissue and is, therefore, unable to reach its target cells under physiologic conditions, a functional effect on the tissue might be mediated by peptide-binding to specific cell surface receptors.

Keywords Human urinary bladder · Peptides

The regulation of human detrusor smooth muscle tone is a complex physiological mechanism that involves the interaction of various transmitters and effector compounds [4, 7, 12]. Urine storage and bladder accommodation are controlled by multiple interactions. The function and distribution of cholinergic, adrenergic and purinergic receptors are well established and it is without doubt that the release of neurotransmitters, such as adrenaline, acetylcholine and adenosine triphosphate (ATP), contribute to the regulation of urinary bladder contraction and relaxation. While the sympathetic nervous system dominates during the filling phase, the excitatory parasympathetic and purinergic system is more dominant during voiding, eliciting contraction of the detrusor. Many other compounds are supposed to have a putative physiological role not as neuromuscular...
transmitters but as modulators, either potentiating or inhibiting cholinergic or purinergic activity. Such modulators include nitric oxide (NO), prostaglandins and various bioactive peptides. These peptides, which have been demonstrated to be synthesised, stored and released in the human lower urinary tract, include atrial natriuretic peptide (ANP), bradykinin, calcitonin gene-related peptide (CGRP), endothelin, enkephalins, galanin, neuropeptide Y, somatostatin, substance P and vasoactive intestinal polypeptide (VIP) [2]. The peptides may be involved in the mediation of smooth muscle contraction or relaxation, as well as in changes in vascular tone and permeability of the urothelial layer. It has also been discussed whether peptides are involved in the pathogenesis of bladder hyperactivity, and results from immunohistochemical studies have provided evidence that chronic obstruction, which is commonly associated with conditions of bladder overactivity, causes a reduction in the density of peptide-containing nerves [6]. Moreover, it is already well established that the release of peptides into the extracellular space is one of the most important mechanisms by which communication between cells and the normal function of mammalian tissues is maintained. Thus, during the last two decades, there has been increasing interest, especially in peptidergic inhibitory mechanisms, in order to clarify which peptides mediate the relaxation of the human urinary bladder [5, 9]. Since evidence of the motoric response of isolated urinary bladder is based mainly on experiments using various laboratory animals, we have examined the effects of ANP, CGRP, ET-1, SP and VIP on isometric tension and tissue levels of cAMP and cGMP of isolated human detrusor smooth muscle strips. Moreover, we determined the presence of those peptides in the human detrusor by means of light microscopy immunohistochemistry.

**Material and methods**

**Organ bath studies**

Macroscopically normal human detrusor from the bladder dome and lateral walls was obtained from male and female patients who had undergone surgery for pelvic malignancies. Square strips preparations were mounted in the chambers of a vertical organ bath system (MAYFLOWER, Hugo Sachs Elektronik GmbH, March, Germany) under standard conditions (KREBS-RINGER bath system (MAYFLOWER, Hugo Sachs Elektronik GmbH, March, Germany) under standard conditions (KREBS-RINGER solution continuously gassed with O₂/CO₂). Tissue strips were incubated with F and SNP (0.01, 1.0 and 100 μM), with 0.01, 0.1, and 1.0 μM of VIP for 2 min and 5 min, and with ANP and CGRP for 10 min. The tissue was then rapidly frozen in liquid nitrogen and homogenised in the frozen state. cAMP and cGMP were extracted from tissue homogenates using 80% ethanol. Following centrifugation, the ethanolic phase was removed and lyophilized and the remaining dry particulate fraction resuspended in 50 mM sodium acetate buffer. Cyclic nucleotides were assayed using specific radioimmunoassays.

**Immunohistochemistry**

**Fixation protocol**

Specimens from normal human detrusor were immediately frozen in isopentan chilled by liquid nitrogen. Specimens were sectioned with a cryostat, mounted on glass slides, and fixed with ice cold acetone for 10 min followed by a mixture of 4% paraformaldehyde and 0.4% glutaraldehyde in 0.1 phosphate buffered saline (PBS) for 1–6 h.

**Immunohistochemical assays**

Primary antibodies (host: rabbit) were diluted 1:10 to incubate the sections (20 μM) overnight at 4°C. To visualise the peptides, a goat-anti rabbit IgG antiserum (dilution 1:200, incubation for 90 min) and the Vectastain ABC Elite Kit (Vector Laboratories, Burlingame, USA) were used according to the instructions of the manufacturer.

**Chemicals**

Anti-ANP, -CGRP, -ET-1, -SP, and -VIP antibodies were obtained from Paesel & Lorei GmbH, Frankfurt, Germany. 125IcGMP were purchased from Amersham-Pharmacia Biotech Europe GmbH (Freiburg, Germany). ANP, CGRP and SP were obtained from Bachem GmbH (Heidelberg, Germany). VIP was obtained from ICN Biomedicals (Costa Mesa, USA). Antibodies raised in rabbits against cAMP and cGMP were generously provided by IPF Pharmaceuticals GmbH (Hanover, Germany). Glutaraldehyde was from Lancaster Synthesis Ltd. (Newgate, UK). All other laboratory chemicals were either from Merck KG (Darmstadt, Germany) or Sigma Chemical Company (St. Louis, USA).

**Results**

**Organ bath studies**

Muscarinic tension was most effectively reversed by adenyl cyclase stimulating dipterene forskolin, non-specific PDE inhibitor papaverine and VIP with the following rank order of potency: F > P > VIP. 0.1 μM VIP of both human and porcine origin reversed the tone to 76 ± 3.6% (porcine VIP) and 82.0 ± 2% (human VIP) of the initial maximum carbachol-induced tension. Thus, 0.1 μM VIP was almost as potent as 1 μM forskolin (EC50 = 6 μM, Rmax = 92 ± 2%), 20 μM papaverine (EC50 = 90 μM, Rmax = 56 ± 9%) and about three-fold more potent than 100 μM SNP. The relaxing effects of ANP and CGRP on detrusor strips were negligible (Rmax < 10%). In contrast, ET-1 and SP induced contraction of the tissue strips, starting at a concentration of 5 nM and 100 nM, respectively. Maximum