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The Effects of Some Neuropeptides on Motor Activity of Smooth Muscle Organs in Abdominal and Pelvic Cavities

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1. Introduction

1.1. Neuropeptides

Neuropeptides are intracellular peptides, composed of short chains of amino acids and found in brain tissue. They are often localized in axon terminals at synapses and are released as intercellular messengers that transmit information in the central nervous system, gastro-intestinal tract etc. Many are also hormones released by nonneuronal cells. Neuropeptides can be divided and grouped according their site of synthesis and secretion or their structural or functional characteristics. Currently recognized neuropeptides include all hypothalamic releasing hormones, pituitary hormones, gastro-intestinal and brain peptides, some circulating hormones, opioide peptides, neurohypophyseal hormones etc (Siegel, 2006). Some neuropeptides are secreted by the nerve terminals with conventional neurotransmitters. But which are the differences between the classical neurotransmitters and the neuropeptides? The precursors of neuropeptides have at least 90 amino acids residues - larger than the precursors of the neurotransmitters. The synthesis of neuropeptides is carried in the neuronal soma and then is transported to the axonal ends. The secretion of neuropeptides requires lower concentration of intracellular Ca^{2+} in comparison to transmitters. After secretion the neuropeptides or their precursors are reused in the synapse. The concentration of the neuropeptides in the tissue is very low and they interact with the receptors at lower concentrations than neurotransmitters. Neuropeptides appearance and secretion are very plastic (Siegel, 2006). For example in pathological conditions, the number of endocrine cells that secrete neuropeptides can not only increase but also appear unusual locations as a result of additional stimulation (Gulubova et al., 2012).

1.2. Vasopressin

Vasopressin (arginine vasopressin, AVP) is the first identified neuropeptide. AVP is a nonapeptide that is synthesized in magnocellular and parvocellular neurons, located in the paraventricular and supraoptic nuclei of the hypothalamus (Swaab et al., 1975). Most of vasopressin is released from the axonal terminals of magnocellular neurons directly into the bloodstream of the posterior pituitary.

The effects of AVP are mediated mainly via V1 and V2 receptors.

V1 receptors are located on the vascular smooth muscle membranes. They are also found in myometrium and urinary bladder smooth muscle cell membranes. V1-receptor activation mediates vasoconstriction by receptor-coupled activation of phospholipase C and release of Ca^{2+} from intracellular stores via the phosphoinositide cascade (Thibonnier, 1992, Briley et al., 1994).

V2 renal receptors are present in the renal collecting duct system and endothelial cells. Kidney V2 receptors interact (by the G protein complex) with adenylyl cyclase to increase intracellular cyclic adenosine monophosphate (cAMP) and cause retention of water (Orloff & Handler, 1967).

V3 pituitary receptors (formerly known as V1b or AVPr1B), have central neural system effects, such as increasing adrenocorticotrophic hormone production, activating different G proteins, and act via increasing intracellular cAMP (Thibonnier et al, 1997, Holmes et al, 2001, Kam et al, 2007).

The classical effects of vasopressin are mainly related to maintenance of water-electrolyte homeostasis and blood pressure. During the last years the data about the effects of this neuropeptide on brain function and behavioral reactions increase. The brain effects of vasopressin can be divided into two main types: those related to its peripheral effects such as hormone and focused on the maintenance of water balance. Others are associated with higher brain functions as learning, memory, emotion and they are independent of its hormonal effects (Frank & Landgraf, 2008). Vasopressinergic axons propelled from hypothalamus to many brain regions as hippocampus, septum, amygdala and brainstem, secrete AVP that acts as neurotransmitter. This extrahypothalamic vasopressin network is an anatomical basis of involvement of limbic-midbrain structure in processes of learning and memory. AVP facilitates consolidation and retrieval of memory (Kovacs et al., 1979)

Vasopressin participates in formation of circadian rhythms and regulation of biological clock. The suprachiasmatic nucleus (SCN) is responsible for generation of circadian rhythmicity in mammalian brain and is an obvious source for a vasopressin innervation of GABAergic neurons located in this area (Hermes et al., 2000). A significant diurnal variation in vasopressin release in the SCN was detected, with the highest levels occurring during midday and a trough around midnight (Kalsbeek et al., 1995). It was demonstrated that melatonin synthesis was stimulated after local injection in pineal gland of vasopressin. Also the night melatonin plasma concentration was increased after prolonged period of water deprivation. These results show that vasopressin can modulate melatonin synthesis in the

rat pineal (Barassin et al., 2000). The suprachiasmatic nuclei, that are the main biological clock, contain vasopressinergic neurons. They demonstrate noticeable daily variation activity. In animals these vasopressin secreting neurons have very important role in the control of day/night rhythms. The reduced secretion of vasopressin in suprachiasmatic nuclei could contribute to the violation of sleep-wake rhythms during ageing and to development of depression (Kalsbeek et al., 2010).

Vasopressin takes part in the regulation of male typical social behaviors, vocal communication, aggression, and paternal care (Goodson & Bass, 2001). There are established projections of vasopressinergic neurons from the cells of the bed nucleus of the stria terminalis to the lateral septum with higher levels of density in nonaggressive animals. These localizations demonstrate the significance of vasopressinergic brain network in development of aggression (Compaan, 1993).

AVP is a potent regulator of complex social maternal behaviors. The maternal cares that are vasopressin dependent were found to be independent of dam's trait anxiety. The authors suggest that manipulation of AVP system could contribute to the treatment of mothers suffering from postpartum depression (Bosch & Neumann, 2008).

Vasopressin, secreted in olfactory bulb is involved in the processing of stimuli that are important for social behaviors. In this anatomic region Tobin and coauthors (2010) have identified population of vasopressin neurons, most of which do not project outside the olfactory bulb. They discuss the importance of vasopressin secreting neurons in filtering out of the olfactory signals and in social recognition (Tobin et al., 2010).

Vasopressin and corticotropin-releasing hormone, secreted from parvocellular portion of paraventricular nucleus in stress condition synergistically activate ACTH-adrenal axis. Aguilera supposed that in chronic stress there is preferential activation of vasopressin rather than corticotropin-releasing hormone and as a consequence a feedback mechanism is disintegrated (Aguilera, 1994). In contrast Zelena et al. (2006) demonstrate that AVP does not play critical role in stimulation of hypothalamic-pituitary axis during chronic stress, but its role in acute stress is more important.

1.3. Ghrelin

Ghrelin is a multifunctional peptide hormone (28 amino acids) secreted from the cells of the diffuse neuro-endocrine system. Ghrelin-secreting cells are found from the stomach to the colon (in the oxyntic glands of the fundus and less in the small and large intestine) (Broglia et al., 2002; Lee et al., 2002; Inui et al., 2004). Ghrelin has been detected in the central nervous system, e.g. in arcuate nucleus and hypothalamus (Lu et al., 2002), in pancreas (Date et al. 2002), in some cells of the immune system (lymphocytes and monocytes) (Mager et al., 2008) and also in human ovaries and testes (García et al., 2007). There is a hypothesis that ghrelin might have not only endocrine but also autocrine and paracrine mechanism of action.

The presence of ghrelin receptor subtype GHS-R1a is detected in hypothalamus (n. arcuatus) and the pituitary gland, in multiple organs with nonendocrine and endocrine function (heart, lung, liver, kidney, pancreas, stomach, small and large intestines, adipose tissue,

immune cells, gonads, thyroid gland, adrenal gland) (Broglia et al., 2003; Inui et al., 2004; Van Der Lely et al., 2004) and in gastrointestinal vasculature (Mladenov et al., 2006). They are also expressed by lumbosacral autonomic preganglionic neurons of the micturition reflex pathways (Ferens et al., 2010)

The activation of the receptor causes the stimulation of the G-protein subunit $G\alpha_{11}$. This leads to the activation of intracellular signaling cascades via the phospholipase C (PLC) (Vartiainen, 2009).

The principal physiological action of ghrelin is the stimulation of secretion of growth hormone. Therefore ghrelin is a hormone with anabolic effect. It participates in the regulation of metabolism, energy homeostasis and feeding behaviors which are mediated via a complex neuroendocrine network (Van Der Lely et al., 2004). Ghrelin increases appetite and food intake and decreases fat utilisation as a metabolic fuel and increases fat storage in the adipose tissue. Ghrelin modulates gastric motility and emptying and gastric acid secretion and stimulates ileum peristalsis, most of these effects being vagally mediated (Ghigo et al., 2005). It induces fasted motor activity in the duodenum (Fujino et al., 2003). Ghrelin activity is mediated via enteric nervous system (Tack et al., 2006).

Ghrelin and its receptors are present not only in the peripheral tissues but also in the central nervous system (Kang et al., 2011). Ghrelin functions as a peripheral hormone that is released mainly from the stomach and affects the hypothalamus, but also as a neuropeptide in hypothalamus (Kojima and Kangawa, 2005). Like other neuropeptides, Ghrelin is widely distributed in the brain in key areas of emotional regulation, and plays role as modulators of behavioural states (Kang et al., 2011). Ghrelin plays an important role in the regulation of energy balance by regulating food intake, body weight, glucose homeostasis and feeding behaviour which are mediated by a complex neuroendocrine network (Kalra et al., 1999; Van Der Lely et al., 2004). The regulation of energy balance is related to somatic growth and instinctive behaviour, including feeding, reproduction and emotion, and is a complex phenomenon involving interaction of the central and peripheral nervous systems, neuroendocrine system and gastrointestinal system (Matsuda et al., 2011). Ghrelin induces in the brain an orexigenic effect, modifies locomotor activity and also is involved in the control of psychophysiological functions and regulation of metabolism (Kang et al., 2011). The hypothalamic region of the brain plays very important role in the regulation of feeding and neuroendocrine functions (Kalra et al., 1999). Many types of neurons in the hypothalamus and related regions express ghrelin and some neuropeptides, such as, orexin, NPY, agouti-related peptide (AGRP), melanin-concentrating hormone (MCH) and other, which are implicated in the regulation of feeding behaviour and also in energy homeostasis in mammals (Eva et al., 2006; Kalra et al., 1999; Pickar et al., 1993). Ghrelin increases orexigenic effect and food intake but decreases energy expenditure thus inducing weight gain. (Kojima & Kangawa, 2005; Van Der Lely et al., 2004). Ghrelin exerts its central orexigenic effect through activation of hypothalamic neurons in the arcuate nucleus, important area involved in the regulation of energy balance and in addition it stimulates the neurons of other areas of the central nervous system, for example nucleus paraventricularis, dorsomedial parts of hypothalamus, and areas in the brain stem nucleus tractus solitarius

and the area postrema, which all take part in the modulation of appetite control (Lim et al., 2010; Vartiainen, 2009). Ghrelin-secreting hypothalamic neurons send efferent fibers onto key circuits involved in the central regulation of energy homeostasis. They balance the activity of orexigenic neuropeptide Y/agouti-related peptide neurons in the arcuate nucleus and the activity of anorexigenic pro-opiomelanocortin (POMC) neurons that secrete alpha melanocyte stimulating hormone (α -MSH) and thus modulate the resultant effect (Van Der Lely et al., 2004).

Several new intracellular targets/mediators of the appetite-inducing effect of ghrelin in the hypothalamus have recently been identified, including the AMP-activated protein kinase, its upstream kinase calmodulin kinase kinase 2, components of the fatty acid pathway and the uncoupling protein 2 (Lim et al., 2010).

Recently, it has been demonstrated that ghrelin plays an important role in the regulation of central and peripheral lipid metabolism through specific control of hypothalamic AMP-activated protein kinase (AMPK), a critical metabolic regulator of both cellular and whole-body energy homeostasis (Kola et al., 2005).

Centrally administered ghrelin has various effects such as arousal, increasing gastric acid secretion and gastrointestinal motility, inhibition of water intake and release of some hormones from the pituitary, mainly growth hormone (Hashimoto et al., 2011).

Ghrelin may be synthesized in the hypothalamus. Ghrelin have hypothalamic actions on growth hormone-releasing hormone neurons (Sun et al., 2007). Ghrelin acts centrally to exert a global stimulatory effect on the hypothalamo-pituitary-adrenal axis. Ghrelin increases absolute whole adrenal gland weight and whole adrenal gland volume and elevates blood concentrations of ACTH, aldosterone and corticosterone (Milosević VLj et al., 2010). Ghrelin may function as a metabolic modulator of the gonadotropic axis, with inhibitory effects in line with its role as signal of energy deficit. These effects likely include inhibition of luteinizing hormone secretion, as well as partial suppression of normal puberty onset (Tena-Sempere, 2008).

Ghrelin-immunoreactive neurons are present in the paraventricular, dorsomedial, ventromedial and arcuate nuclei, areas important for circadian output. Contrary to the effects of ghrelin on appetite, growth hormone release and the sleep-wake cycle, little is known about the effects of ghrelin on circadian rhythms (Yannielli et al., 2007).

Central ghrelin is also a gastroprotective factor in gastric mucosa. The gastric protection elicited by central ghrelin requires integrity of capsaicin-sensitive sensory neurons, which play an important role in gastric cytoprotection. Growing evidence indicates that the mechanisms triggered by peptides to increase resistance of the gastric mucosa involve changes in the release of gastric protective factors. Endogenous prostaglandins are involved in ghrelin gastroprotection (Sibilia et al., 2008).

The short-term activation of AMPK in turn results in decreased hypothalamic levels of malonyl-CoA and increased carnitine palmitoyltransferase 1 (CPT1) activity. Ghrelin deficiency induces reductions in both de novo lipogenesis and beta-oxidation pathways in

the hypothalamus. There are reductions in fatty acid synthase (FAS) mRNA expression both in the ventromedial nucleus of the hypothalamus and whole hypothalamus, as well as in FAS protein and activity. CPT1 activity is also reduced. Chronic ghrelin treatment does not promote AMPK-induced changes in the overall fluxes of hypothalamic fatty acid metabolism in normal rats and this effect is independent of ghrelin status. In addition, ghrelin plays a dual time-dependent role in modulating hypothalamic lipid metabolism. (Diéguez C et al., 2010; Sangiao-Alvarellos et al., 2010)

A reciprocal relationship exists between ghrelin and insulin, suggesting that ghrelin regulates glucose homeostasis (Sun et al., 2007).

1.4. Angiotensin II

The octapeptide Angiotensin II (Ang II) is the main effector of the renin-angiotensin system (RAS). Ang II is generated in circulation or locally in tissues in the kidney, blood vessels, heart, and brain and etc.

The signal transduction mechanism for AT1 receptors is well known. AT1 receptors are distributed in adult tissues including blood vessel, heart, kidney, adrenal gland, liver, brain, and lung. These receptors activate phospholipase A2, phospholipase C, phospholipase D and L-type Ca^{2+} channels and inhibiting the adenylyl cyclase (Shokei & Hiroshi, 2011).

AT2 receptors are ubiquitously expressed in developing fetal tissues, suggesting a possible role of these receptors in fetal development and organ morphogenesis. AT2 receptors expression rapidly decreases after birth, and in the adult. Expression of these receptors are limited mainly to the uterus, ovary, certain brain nuclei, heart, and adrenal medulla. In various cell lines, AT2 receptors activated protein tyrosine phosphatase was shown to inhibit cell growth or induce programmed cell death (apoptosis) (Kim and Awao, 2011).

Ang II has a multifunctional role. It is general regulator of salt and water metabolism, thirst, sympathetic outflow and vascular smooth muscle cell tone. As a result Ang II acts as a principal controller of long term regulation of blood pressure (Robertson, 2005; Watanabe et al., 2005). Later, Ang II was found to exert long-term effects on tissue structure, including cardiac hypertrophy, vascular remodeling, and renal fibrosis (Watanabe et al., 2005).

The key effector of peripheral renin-angiotensin system (RAS) - Ang II in circulation does not cross blood-brain barrier. Therefore it interacts on brain regions that lack the blood-brain barrier as circumventricular areas, organum vasculosum laminae terminalis, where Ang II stimulates salt appetite, thirst and vasopressin secretion (Fitts et al., 2000). Also, it influences neuronal activity in area postrema and takes part in central regulation of blood pressure (Otsuka et al., 1986).

Many immunohistochemical studies demonstrate the distribution of all components of RAS - angiotensinogen, Ang I, Ang II and renin in several brain regions of rats. Immunoreactivity for Ang II was detected in neurons and vessels in the brainstem, cerebellum, hypothalamus, basal ganglia, thalamus and cortex while for angiotensinogen and Ang I were found in

neurons of the hypothalamic nuclei in rats (McKinley et al., 2003; Von Bohlen et al., 2006). AT1 receptor binding sites with higher density were localized in the lamina terminalis, hypothalamic paraventricular nucleus and the nucleus tractus solitaries, lamina terminalis and the subfornical organ. The median preoptic nucleus also contains membranes rich in AT1 receptors. All regions that are included in the regulation of cardiovascular functions as caudal ventrolateral medulla, and the midline raphe, also have AT1 receptors (Allen et al., 1999; Lenkei et al., 1997). In the midbrain -in the lateral parabrachial nucleus, substantia nigra and periaqueductal gray AT1 receptors are presented from moderate to high densities (Lenkei et al., 1997). These localizations of RAS brain components show that Ang II is involved in the regulations of thirst, drinking, facilitating vasopressor effects and secretion of vasopressin, adrenocorticotrophic and luteinizing hormones. Ang II also stimulates secretion of neurotransmitters such as noradrenaline and 5-hydroxytryptamine (5-HT) and inhibits acetylcholine release. The brain RAS appears to be also an important modulator of the blood pressure circadian rhythm and it influences renal renin release.

Recent findings demonstrate that central effects of Ang II contribute to facilitated learning and enhance associative memory and learning possibly with differential effects on acquisition, storage and recall. Brain RAS is involved in the development of affective disorders and Ang II has a modulating effect of on anxiety (Georgiev & Yonkov, 1985).

RAS receptors alterations have been found in some neurodegenerative disorders - Parkinson's and Huntington's disease (Ge & Barnes, 1996). The number of AT1 receptors in caudate nucleus, putamen and substantia nigra was significantly decreased in Parkinson's disease patients in comparison to controls. In Huntington's disease patients, AT1 receptors was found to be slightly decreased in putamen (Ge & Barnes, 1996). AT2 receptors that are localized in caudate nucleus was decreased in Parkinson's and increased in Huntington's disease patients. The receptor alterations were considerable; therefore the authors have concluded that brain RAS seems to decisively contribute to the pathology of the dopaminergic nigrostriatal pathway in these patients and may be a novel therapeutic target for neurodegenerative disorders (Savaskan, 2005).

1.5. Cholecystokinin

In 1928 Ivy and Oldberg extracted from dog duodenal mucosis a substance which injected intravenously contracted the gallbladder. The authors named this substance cholecystokinin (CCK). Later, Harper & Raper (1943) found a compound in this extract that stimulated the pancreatic secretion and called it pancreozymin. Purifying both hormones and determining their amino-acid sequection, Mutt (1980) proved them to be the same linear polypeptide, containing 33 amino-acid residues, and proposed the hybrid name "cholecystokinin-pancreozymin". Different CCK forms have been shown to exist: CCK-58, CCK-39, CCK-33, CCK-27, CCK-12, CCK-8, CCK-4, all of them containing a bioactive C-terminal. CCK-58 and CCK-39 are precursors of CCK-33 and by the degradation of the latter the shorter forms are obtained. Cholecystokinin octapeptide (CCK-8) is the most active one and is most widely spread in the gastro-intestinal tract and in the central nervous system. A cholecystokinin analogue, named caerulein, has been isolated from the skin of the frog *Hyla caerulea*.

CCK and gastrin possess identical 5 aminoacids at their C-terminals that are the biologically active part of both hormones. The dissimilarities in their potency and physiological activity are determined by the different positions of the *Tyr*-residue in the molecules of both peptides. When the *Tyr*-residue is in the 6th position, the peptide (gastrin) strongly potentiates the gastric secretion, its stimulating effect on the gallbladder contractions and pancreatic secretion being much weaker. With the *Tyr*-residue in the 7th position, CCK markedly enhances the gallbladder motility and the pancreatic secretion.

Immunohistochemical studies have shown that CCK is synthesized in the mucosal endocrine cells type I and type K of the small intestine, and in the endocrine cells type A of the pancreas. CCK-immunoreactivity has been also identified in the vagus nerve. Cholecystokinin is so called “brain-gut” neuropeptide – it is also produced by enteric neurons, and is widely and abundantly distributed in the brain.

The food intake in the small intestine is the main physiological stimulus for the CCK release – masts, proteins and aminoacids are the most powerful stimulants among the foods. The plasma CCK concentration increases from 1-2 pmol/l to 6-8 pmol/l after feeding (Cantor, 1986). Cholecystokinin plays a key role in facilitating digestion within the small intestine – this peptide stimulates delivery into the small intestine of digestive enzymes from the pancreas and bile from the gallbladder. Recently it was shown that CCK-8 can reduce food intake by capsaicin-insensitive, nonvagag mechanisms (Zhang & Ritter, 2012).

Mechanisms of secretion of cholecystokinin group peptides into the gastro-intestinal tract are as follows: a) Endocrine mechanism – the peptide is released by the endocrine cell in a blood vessel and is afterwards transported by the circulation to the effector cell; b) Paracrine mechanism – the peptide is released by the endocrine cell in the intracellular space, reaching afterwards the effector cell by diffusion; c) Neurotransmitter mechanism – the peptide is released by the nerve terminal in the synaptic cleft and affects afterwards the activity of the effector neuron; d) Neuroendocrine mechanism – the peptide is released by the neuron in a blood vessel.

The peptide hormone CCK realizes its effects via binding to specific receptors localized on the cell membranes of the target organs. Two types of cholecystokinin receptors have been characterized so far: CCK_A and CCK_B which are approximately 50 % homologous (Dufresne et al., 2006).

CCK_A (gastro-intestinal) receptors. They prevail in the peripheral target organs (pancreas, gallbladder, small intestine), as well as in the vagus nerve afferent fibres, mediating the pancreatic enzyme secretion and the gallbladder and ileum motility (Crawley & Corwin, 1994; Xu et al., 2008). CCK_A-receptors have also been identified in some brain regions where they take part in the modulation of dopaminergic neurotransmission, and in the regulation of food behavior - satiety effect (Lieverse et al., 1995).

CCK_B (brain) receptors. They have been identified in various brain structures, as their number is largest in the cortex, hippocampus and limbic structures (Hokfelt et al., 1985). CCK_B receptors, similar or identical to the peripheral gastrin receptors, have been demonstrated in peripheral organs, too.

Cholecystokinin is a major peptide hormone in the gut and a major peptide transmitter in the brain. Its synthesis requires endoproteolytic cleavage of proCCK at several mono- and dibasic sites by prohormone convertases. On one hand cholecystokinin is a classical gut hormone and a growth factor for the pancreas. On the other, the CCK gene is expressed also in large quantities in cerebral and peripheral neurons from where CCK peptides are released as potent neurotransmitters and modulators. Accordingly, cerebral CCK defects have been associated with major neuropsychiatric diseases such as anxiety, schizophrenia and satiety disorders (Crawley & Corwin, 1994; Liddle, 1997).

CCK is also a key component of the aggression facilitating circuitry in the brain (Luo et al., 1998), and it is released during inter-male fighting (Becker et al., 2001). In addition to its many aversive motivational/emotional effects, CCK also plays a role in more positively valenced motivational states, such as mating (Dornan & Malsbury, 1989; Markowski & Hull, 1995), drug addiction (Crespi, 2000) and brain reward processes (Degen et al., 2001, Josselyn, 1996).

It was demonstrated that CCK is colocalized with dopamine in ventral striatal dopamine neurons (Hokfelt et al., 1980). Consistent with the neuronal colocalization and extensive overlap of expression between CCK and the dopaminergic system, CCK peptides have significant effects on dopamine mediated behaviors. Administration of CCK peptides exhibit many of the behavioral characteristics of antipsychotics including inhibition of conditioned avoidance responding (Cohen et al., 1982), inhibition of apomorphine-induced stereotypic behavior (Zetler, 1983), and inhibition of amphetamine-induced hyperlocomotion (Schneider et al., 1983). Microinjection of CCK into the anterior nucleus accumbens inhibits dopamine release, inhibits dopamine-mediated behaviors and is blocked by a CCK_A antagonist whereas injection into the posterior nucleus accumbens has the opposite effects and these effects are mediated by CCK_B receptors (Vaccarino & Rankin, 1989, Crawley, 1992). Thus, it appears that different CCK-based circuitries in the brain can facilitate both negative and positive emotional processes. It is also interesting to note that selective CCK_B agonists that cross the blood brain barrier such as pentagastrin and CCK-4 are used to induce panic attacks in clinical studies. Consequently, a CCK agonist for schizophrenia would need to be either nonselective or CCK_A selective.

CCK was the first gut hormone discovered to have anorectic effects. Its actions include inhibition of food intake, delayed gastric emptying, stimulation of pancreatic enzyme secretion, and stimulation of gall bladder contraction. These effects are mediated via binding to CCK receptors on the vagus nerve. CCK administration to humans and animals inhibits food intake by reducing meal size and duration. However, at high dose nausea and taste aversion have been detected making CCK an unlikely candidate for an anti-obesity treatment.

2. Materials and methods

Wistar rats of both sexes weighting 200–250 g were used. The experiment was carried out in accordance with the national regulations and DIRECTIVE 2010/63/EU of the European Parliament and of the Council of 22 September 2010 concerning the protection of animals

used for scientific and experimental purposes. The animals were anesthetized with Nembutal 50 mg/kg intraperitoneally and exsanguinated. Abdominal cavity was opened and longitudinal strips from different parts of gastro-intestinal tract, urinary bladder and uterus horn were dissected out. The isolated organs were transferred immediately in cold Krebs solution 3 °C), containing following composition in mM): NaCl—118.0, KCl—4.74, NaHCO₃—25.0, MgSO₄—1.2, CaCl₂—2.0, KH₂PO₄—1.2 and glucose 11.0.

Longitudinal strips approximately 2 mm wide, 0.5 mm thick and 8 mm long) were dissected following the direction of the muscle bundles. The two ends of each strip were tied with silk ligatures. The distal end was connected to the organ holder; the proximal end was stretched and attached to a mechano-electrical transducer FSG-01 (Experimetria, Ltd., Hungary) via a hook. The preparations were mounted in organ baths TSZ-04/01, containing Krebs solution, pH 7.4, continuously bubbled with Carbogen (95% O₂, 5% CO₂). The organ baths were mounted in parallel above an enclosed water bath, maintaining the solution temperature at 37 °C. Each smooth muscle strip was initially stretched to a tension of 1.0 g followed by 90 minutes of equilibration. During this period, the smooth muscle strips were replenished with fresh Krebs solution at 15-th min, 60-th min and 75-th min.

After initial period of adaptation, they were treated with the solution of different neuropeptides, and the obtained responses were registered.

The phasic contractions of the smooth muscles before application of neuropeptide and the changes of motor activity, expressed as tonic contractions, relaxations or lack of reaction after treatment were recorded. The contractile activity signals were transduced by mechanical-force sensor, amplified, digitized and recorded using ISOSYS ADVANCED digital acquisition software, produced by Experimetria Ltd., Hungary.

Chemicals and drugs

Ang II (Sigma-Aldrich), vasopressin (Sigma-Aldrich), ghrelin (PolyPeptide Group) synthetic octapeptide of cholecystokinin (Squibb, USA), acetylcholine chloride (Sigma-Aldrich), hexamethonium chloride (Sigma-Aldrich) were solubilized in bidistilled water. All reagents for the preparation of Krebs solution were purchased from Sigma-Aldrich.

Data analysis and statistic processing

The recorded force-vs.-time curves permit determination of amplitudes and integrated force of contraction, the latter represented by the area under the curve (AUC), as well as defining of time parameters. Data acquisition and the initial conversion of the experimental data for the later analysis was performed with KORELIA – Processing software (Yankov, 2010). For later analysis, evaluation and identification was used KORELIA-Dynamics program. (Yankov, 2006; Yankov, 2011).

Following time parameters were examined Figure 1:

- T_{hc} (s) – first half contraction time - time interval between the start of the smooth muscle contraction (SMC) and half-contraction moment ($T_{hc} = t_{hc} - t_0$);

- $T_{(c-hc)} (s)$ – second half contraction time - - time interval between the end of T_{hc} and maximum peak of the SMC ($T_{(c-hc)} = t_{peak} - t_{hc}$)
- $T_c (s)$ – contraction time - time interval between the start of the SMC and the moment of the maximum peak ($T_c = t_{peak} - t_0$);
- $T_{hr} (s)$ – half-relaxation time: time interval between the moment of the maximum peak t_{peak} and the moment when the curve decreases to $F_{max}/2$ ($T_{hr} = t_{hr} - t_{peak}$);
- $T_{chr} (s)$ – contraction plus half-relaxation time: time between the start of the SMC and t_{hr} ($T_{chr} = t_{hr} - t_0$).

Figure 1. Smooth muscle contraction (SMC) - graph and parameters. F_{max} – maximal force of the SMC; $F_{max}/2$ – half of maximal force of the SMC; t_0 – start of SMC. $t_0 = 0$; t_{hc} – half-contraction moment; t_{peak} – the moment of the maximum peak F_{max} ; t_{hr} – the moment when the curve decreases to $F_{max}/2$.

The duration of the tonic contraction was defined from the beginning of the contraction, until the amplitude fell to 50%.

For a more correct and accurate comparison between the parts of the gastrointestinal tract was performed normalization of the different intervals as a relative part of total length of the process $T_{xn} = T_x / T_{chr}$. As a result the following normalized parameters were obtained: $T_{hc/n}$, T_{cn} , $T_{(c-hc)/n}$, $T_{hr/n}$.

The gall bladder pressure *in vivo* was recorded in six conscious dogs weighing 18 to 20 kg. The animals were starved for 18 hours and were then anesthetized with chloralose (90 mg/kg i.v.). Laparotomy was performed through upper midline abdominal incision. Gall bladder bile was aspirated with a syringe. The bile volume was between 30 and 40 ml. A small balloon mounted on the top of a polyethylene catheter was introduced into the gall bladder. Experiments were started four weeks after surgery and were carried out twice a week at of at least two-day intervals. The dogs were deprived of food, but were given water *ad libitum* for 18 hours before each trial. The balloon was filled with 1 ml distilled water and connected through a catheter to a pressure transducer. The changes in gall bladder pressure were measured in mmHg. The first 40-min records were used as controls. Cholecystokinin

octapeptide was then injected i.v. at increasing doses every 30 min. Atropine or hexamethonium was administered before cholecystokinin.

Obtained data were processed by the statistical program Statistica 6.1, StaSoft, Inc. and presented as mean $\pm$ standard error. A P-value less than or equal to 0.05 was considered to be statistically significant.

3. Results and discussions

3.1. Urinary bladder

The coordination of smooth muscle activity of the urinary tract in the process of the urine evacuation is regulated by complex, and not yet fully understood interactions between neural and hormonal control mechanisms (Dixon et al., 1997).

The urinary bladder is innervated by three groups of peripheral nerves: sacral parasympathetic (Helm et al., 1982; Crowe & Burnstock, 1989; Gabella & Uvelius, 1990; Lasanen et al., 1992; Uvelius & Gabella, 1998), thoracolumbar sympathetic (Downie, 1981; Feher & Vajda, 1981) and sacral sensory (De Groat & Booth, 1993). According to the secreted mediator in the neural terminals innervation is classified as cholinergic, adrenergic, and non-adrenergic, non-cholinergic (NANC) (Callahan & Creed, 1986). In humans detrusor neurotransmission is exclusively cholinergic (Andersson et al., 1982; Sibley, 1984; Chen et al., 1994), while its adrenergic innervation is sparse, nonuniform, and it is considered non-essential for micturition function (de Groat & Booth, 1984; Janig & McLachlan, 1987).

A lot of different neuropeptides have been found to be synthesized, stored and released from organs in the lower urinary tract (LUT). Some of them are secreted from the peripheral neural terminals of the autonomic nervous system – vasoactive intestinal peptide (VIP), tachykinins (substance P), neuropeptide Y, calcitonin gene-related peptide, neurokinin A. Others which act by para- and autocrine mechanisms as angiotensin II are locally synthesized. The exact function of many of these peptides has not been fully established, however they may play role in a sensory and efferent innervation (de Groat & Kawatani, 1985; Burnstock, 1990; Maggi, 1991) or serve as neuromodulators in the ganglia or at the neuromuscular junctions. Their actions have been thought to include mediation of the micturition reflex activation, smooth muscle contraction, potentiation of efferent neurotransmission and changes in vascular tone and permeability (Maggi, 1991, 1995; Hernandez et al., 2006).

A few hormones with systemic circulation – vasopressin, oxytocin, are influencing bladder function (Uvelius B. et al., 1990; Romine & Anderson, 1985). These also include a newly discovered peptide ghrelin, which is secreted from the gastrointestinal tract, and stimulates contractions of smooth muscles of blood vessels and gastro-intestinal tract (Tack et al., 2006; Wiley & Davenport, 2002)

Angiotensin II and urinary bladder

There is insufficient information on the effect of Ang II on non-cardiovascular organ smooth muscles (Touyz & Berry, 2002). The interactions of the Ang II with the urinary bladder are of

particular interest regarding the genesis and treatment of the disorders of the micturition. The physiological effects of Ang II on the function of the urinary bladder and the transduction mechanisms which mediate it have not been fully elucidated. Ang II and its precursor Ang I cause dose-dependent contractions of muscle strips from rat urinary bladder (Andersson et al., 1992). According to experimental data of Anderson and co-authors Ang II acts as a modulator in neurotransmission in the urinary bladder (Andersson & Arner, 2004). There are research data confirming that Ang II carries out its physiological effects by binding to membrane AT1 receptors (Tanabe et al., 1993), whose number on the surface of detrusor smooth muscle cells is regulated by the dietary content of sodium and potassium and the age of experimental animals (Weaver-Osterholtz et al., 1996; Szigeti et al., 2005). AT1 receptors activate PLC, dihydropyridine-sensitive Ca^{2+} -channels and inhibit adenylyl cyclase, thus reducing intracellular cAMP concentration (Chiu et al., 1994).

Our experiments show that the administration of Ang II solution to the smooth muscle strips induce tonic contractions, in confirmation of the findings from other researchers concerning its effect on urinary bladder contractile activity. The increased amplitude of contraction following the administration of Ang II in the presence of increased extracellular Ca^{2+} provided evidence of additive synergism (Hadzhibozheva et al., 2009; Tolekova et al., 2010). The blockade of AngII-induced tonic contraction after the administration of blockers of T-type Ca^{2+} -channels unequivocally showed the role of transmembrane Ca^{2+} -influx in the initiation of smooth muscle contraction (Ilieva et al., 2008). The Ang II bindings to its membrane receptors, leads to activation of phospholipase C, which results in formation of inositol triphosphate, which triggers release of Ca^{2+} from sarcoplasmatic reticulum (SR). It's also well known that Ang II causes calcium-induced calcium release in smooth-muscle cells. Angiotensin II causes depolarization and opening of VDCC, providing additional Ca^{2+} influx from the extracellular fluid (Seki et al., 1999). When moving inside the cell, Ca^{2+} binds to ryanodine receptors and triggers supplementary Ca^{2+} release from SR stores (Berridge, 2008). In the experiment we applied specific inhibitor to this particular calcium-induced Ca^{2+} release mechanism. The resulting lack of tonic contraction suggested that this signaling pathway, leading to intracellular calcium increase is of greater importance for the development of detrusor muscle contraction than the inositol triphosphate pathway. Our experimental data also showed that the increase of calcium in extracellular fluid produced additive synergistic effect on Ang II-mediated contraction of detrusor smooth muscle strips.

The circulating AngII is formed in blood under influence of angiotensin-converting enzyme (ACE). During the last decade a lot of new facts that significantly broaden our knowledge of the RAS have been accumulated. Local tissue RAS was found in the blood vessels, heart, kidneys, small intestines, pancreatic tissue, liver, ovaries and brain (Paul et al., 2006). Other enzymes involved in RAS and physiologically active metabolites of Ang I and Ang II were also found (Waldeck et al., 1997; Miyazaki & Takai, 2006).

A lot of recent studies have shown that Ang II acts as a cytokine and growth-like factor. (Kim & Iwao, 2000; Touyz & Berry, 2002). It regulates the smooth muscle mass in the bladder wall in normal and pathological conditions. Chronic bladder outlet obstruction causes changes in smooth muscle mass and connective tissue both in humans' disease and

under experimental conditions in rats (Yamada et al., 2009). The application of ACE blocking substances significantly reduce the quantity of the newly synthesized collagen in the bladder, which is an indirect indicator of the effects of the local RAS on developing pathologic hypertrophic changes in the bladder. Phull and coauthors (2007) showed that applying angiotensin receptor antagonists, reduced urethral resistance on rat models with stress urinary incontinence.

The all main components of RAS – Ang I, Ang II and ACE are found inside bladder tissues (Weaver-Osterholtz et al., 1996). Tissue levels of Ang I and Ang II were higher than circulating levels, which confirms the existence of local synthesis in the urinary bladder, despite the lower measured activity of the angiotensin-converting enzyme, *in situ* is relatively low. This fact supports the hypothesis of auto- and paracrine actions of Ang II. It has been shown that Ang I also causes contraction of smooth muscle cells of the bladder, through the interaction with AT1 receptors.

Ang II -mediated contraction is not completely blocked after administration of ACE inhibitor (Lindberg et al., 1994). These facts indicate the existence of alternative pathways for the synthesis of Ang II. Urata and co-authors show that in the heart the main enzyme converting Ang I to Ang II is a serine protease human himase (Urata et al., 1990). Andersson and co-authors found that application of enalaprilat not fully block contraction of urinary bladder detrusor strips induced by Ang I (Andersson et al., 1992). This means that the remaining contractile effect is due to separate mechanism of Ang II formation.

Vasopressin

Besides its vasoconstrictor activity, AVP is involved in the modulation of the intrinsic smooth muscle activity of the urinary system. Vargiu and coauthors demonstrate that AVP increases the contractile activity of upper urinary tract that is most pronounced in small calices, which are the main pacemaker of the urinary tract and is mediated by V1 receptors (Vargiu et al., 2004). It remains unaffected by the blockade of sodium channels with tetrodotoxin. However, the application of nifedipine and L-type calcium channels blockers reduced spontaneous and AVP-induced activity of smooth muscles of the upper urinary tract (Vargiu et al., 2004). This demonstrates the importance of transmembrane calcium influx for the contractile activity of smooth muscle of the lower urinary tract.

There is a data that shows the presence of V1-receptors in smooth muscle of the urinary bladder, whose binding to AVP leads to activation of the inositol-triphosphate (IP₃) pathway, similarly to binding to Ang II (Crankshaw, 1989; Dehpour et al., 1997). It was found that the removal of extracellular calcium prevents the effects of AVP, suggesting its possible involvement in the activation process (Crankshaw, 1989). In our experiments AVP application to the bladder detrusor smooth muscle strips stimulates powerful tonic contractions. This result supports currently available information on this issue (Uvelius et al., 1990).

Comparison of the effects of Ang II and AVP on contractile activity

Ang II and AVP, accomplish their effects through the formation of the second messenger IP₃. The comparison of the independent effects of Ang II and AVP on urinary bladder strips

shows contractions with approximately equal amplitude, but with integrated muscle force significantly increased after the application of AVP (Figure 2, Table 1) (Tolekova et al., 2010).

	Fmax.g)	AUCgs)	T _{hc} s)	T _{c-hc}	T _c s)	T _{hr} s)	T _{chr} s)
AVP	1,55±0,16	761,29±113,3	28±4	99,6±15	127,4±18	255,3±35	382,7±43
Ang II	1,73±0,26	115,13±20,7	12,6±1,6	19,7±5	32,3±3,3	61,5±13,6	93,7±13,3

Table 1. Force and time-parametes means±SE) of Ang II- and AVP-induced contractions.

Figure 2. Angiotensin II- and AVP – induced contractions after processing of signals with specialized software.

For detailed study and comparison of the tonic contractions under the influence of the two peptides, we are using time parameters, described in Yankov (2011) (Figure 1, Table 1), generated after signal processing with specialized software (Yankov, 2010). The similar parameters were used for investigation of the contractions of the skeletal muscles (Raikova & Aladjov, 2004). Our research results show that Ang II causes contractions with a shorter duration (shorter time for reaching Fmax/2 and Fmax). The AVP-induced response reaches significant AUC value at the expense of the lower rate of increased and decreased contractile activity (longer duration of Thr and Tchr). The greater length of AVP contraction time is mainly due to longer T_(c-hc) period. It is particularly interesting that despite acting through the same transduction pathways, Ang II and AVP cause tonic contractions with different measured force. We assume that this difference, especially the higher speed of relaxation, might be related to the specific Ang II metabolism in bladder cells. Production of Ang III and Ang 1-7 may be a cause of the faster rate of relaxation (Varagic et al., 2008). The interactions of the two peptides – Ang II and AVP with the ion channels of the smooth

muscle membrane may contribute additionally to the differences in the computed parameters and shape of the contraction graphic. There are existing data about the effect of AVP on the potassium channels of brain cells after fluid percussion brain injury indicating that AVP inhibited activity of the KATP and KCa channels (Armstead, 2001). It is known that the urinary bladder smooth muscle cells have a number of potassium channels, including ATP-dependent K channels and Ca^{2+} -dependent K channels (Petkov et al., 2001). Interaction with those channels could be a possible explanation for the prolonged duration of AVP action on smooth muscle contractions.

On the other hand, Ang II stimulates the activity of L/T-type voltage dependent calcium channels in vascular smooth muscle cells (Lu et al., 1996). We can suggest that in the smooth muscle cells of the rat bladder a similar effect takes place.

Role of extracellular calcium for Ang II- and AVP-mediated contractions of smooth muscle cells

The increase of concentration of the extracellular Ca^{2+} exerts a synergistic effect on Ang II- and AVP-mediated contractions. The raise of the amplitude of contraction is a consequence of increased transmembrane calcium influx due to the higher electrochemical gradient. As a result the intracellular calcium concentration is maintained at the higher level than the level of the resting state. There is evidence that this pattern of variations in calcium concentration contributes to the development of the mechanism of "locking" of the smooth muscle cells (Tanaka et al., 2008). We suppose that the above mentioned significant difference in AUC is due to the manifestation of this mechanism (Tolekova et al., 2010).

Ghrelin

The endocrine effects of the peptide ghrelin on various organs and systems are not well examined; however it is known that it stimulates the motility of digestive tract (Tack et al., 2006). On the vascular smooth muscle it exercises a dilatatory influence which is comparable to that caused by adrenomedulline (Wiley & Davenport, 2002). Binding of ghrelin to the its membrane receptors in some tissues triggers signal transduction mechanism via Gq protein and results in activation of PLC and release of IP_3 and Ca^{2+} (Davenport et al., 2005). There are no data in the literature regarding the effects of ghrelin on urinary bladder smooth muscle. The presence of ghrelin receptors on the membranes of detrusor smooth muscle cells is not proven yet. Therefore it is interesting to investigate whether and how ghrelin affects the bladder detrusor and if so by which signal transduction mechanism. Moreover, there is not existing published comparison between the effects of AVP and Ang II on detrusor contractile activity as well as effects of calcium and ghrelin on the smooth-muscle contractions mediated by these peptides.

Does Ghrelin have an effect on a urinary bladder?

The receptors for ghrelin described in the literature mediate their activity with activation of PLC and subsequent increase in concentration of intracellular calcium (Davenport et al., 2005). Therefore, the application of ghrelin on muscle strips of urinary bladder would lead to the occurrence of tonic contractions. During the experiments we found no statistically significant changes in contractile activity after application of ghrelin as compared to the

spontaneous activity. The effects of ghrelin are displayed only when it is applied in combination with other peptides – Ang II or AVP. In combination with Ang II, ghrelin reduces its contractile effect on the bladder (Ilieva et al., 2008, a). The combination of ghrelin with AVP leads to similar yet significantly less manifested decrease, especially in the AUC.

Based on these results we can assume that the urinary bladder possesses receptors for ghrelin, different from those in the digestive tract, with respect to the intracellular signaling mechanism to which they are coupled. The significant reduction in the amplitude of Ang II-induced contraction as well as the partial reduction of AVP-provoked contraction after ghrelin application could be explained by the interaction between signal transduction pathways by which the both peptides act. To our knowledge, this is the first *in vitro* study demonstrated the inhibitory effect of ghrelin on bladder motor activity. Our results were confirmed by Matsuda et coauthors (2011). They showed in experiments *in vivo* that intracerebroventricular administration of Ghrelin increases bladder capacity dose dependently.

It is likely that ghrelin acts through the second messenger cAMP. Activation of this signal pathway causes relaxation of smooth muscles by decreasing the activity of miosinkinase and stimulating Ca^{2+} -efflux. This effect of ghrelin could be explained with interactions between the two types of transduction pathways, which have opposite effects (Rasmussen & Rasmussen, 1990; Churchill, 1985).

3.2. Gastro-intestinal tract

Angiotensin II

Angiotensin II has potent contractile effect on smooth muscles in the gastro-intestinal tract (GIT). The question for the exact effects of Ang II on GIT remains still opened. Local RAS or parts of it had been found in rat rectum (De Godoy et al., 2006), rat small intestine, and in the guinea pig gall bladder (Leung et al., 1993). The role of Ang II had been confirmed in the development of diseases such as the gastro-esophageal reflux (Fändriks, 2010), incontinence of internal anal sphincter (De Godoy et al., 2006; Rattan et al., 2003), and Crohn's disease (Fändriks, 2010; Wang et al, 1993) as well as other inflammatory and motility disorders of the GIT (Fändriks, 2010).

Most of the effects of Ang II concerning the smooth muscle contractile activity of GIT are associated with AT1 receptors (Fändriks, 2010; Fan et al., 2002; Hawcock & Barnes, 1993; Rattan et al., 2003). AT2 receptors are also described in GIT (Fändriks, 2010; Fan et al., 2002; De Godoy et al., 2006; Hawcock & Barnes, 1993; Ewert et al., 2003; Leung et al., 1993; De Godoy et al., 2002). Although different signaling pathways have been assumed, for example activation of various phosphatases, cGMP -NO system etc. (Ewert et al., 2003; Dinh et al., 2001), their actual signal transduction is not quite elucidated. AT2 receptors are associated with the exchange of water and salts, sodium hydrogen carbonate secretion in the duodenum (Fändriks, 2010) and the secretion of nitric oxide in pig's jejunum (Ewert et al., 2003). The significance of AT2 receptors for GIT motility has not been established yet. It is supposed that they have the opposite effect of AT1 receptors (Gallinat et al., 2000), but as a

factor for the smooth muscle relaxation they had been proved only for the internal anal sphincter (De Godoy et al., 2006; De Godoy & Rattan, 2005).

There is not enough information in the literature, regarding to the comparative characteristics of Ang II - induced responses from the various segments of GIT. Dose - dependent curves, which are commonly used as a method for studying the provoked smooth muscle contractions (Fändriks, 2010; Fan et al., 2002; Hawcock & Barnes, 1993; Leung et al., 1993; Park et al., 1973), could give information about the effective doses and maximal responses, but not a data for other important characteristics of the smooth muscle contractions. The different phases of the contraction in the various segments of the GIT, were not clarified and analyzed by application of a time-parameter analysis, as it was made in the study of the skeletal muscle contraction (Raikova & Aladjov, 2004). For comparison and detailed analysis of Ang II contractile effects of Ang II on the different segments of longitudinal strips from rat GIT we are using again time-parameters.

The amplitudes and integral muscle force of different segments from GIT in our experimental study showed marked correlation ($r=0.88$, $p<0.005$). The duodenal muscle strip demonstrated the lowest amplitude and smallest integral force of contraction - 0.55 ± 0.13 g, 41.43 ± 15.52 gs. The amplitudes of the registered angiotensin II-induced contraction from stomach (1.14 ± 0.13 g), jejunum (1.11 ± 0.14 g) and ileum (1.09 ± 0.16 g) are similar and there are not statistically significant differences between them. But stomach integral force (178.09 ± 19.63 gs) is significantly greater than that of duodenum and other intestines and is equally powerful as that of the colon. Under influence of Angiotensin II, rectum developed highest amplitude of contraction 4.74 ± 0.65 g and most powerful integral force - 328.43 ± 75.23 gs.

The analysis of time parameters of Ang II-mediated contractions indicated that the gastric response to Ang II required more time to develop: the time to reach T_{hc} and T_c parameters was 29.09 ± 2.53 s and 78.18 ± 5.87 s respectively. This tendency for a slower progress of the reaction was maintained during the whole contraction of the stomach and its T_{chr} was 224.90 ± 18.45 s. All of the registered intestinal contractions showed similar values for T_{hc} and T_c parameters. For the remaining two time - parameters - T_{hr} and T_{chr} , the results from the intestinal contractions were analogous, with exception of the ileac contraction, which T_{hr} (106.33 ± 9.89 s) and T_{chr} (141.08 ± 9.48 s) were significantly prolonged. After normalization of time-parameter, it was shown that jejunum and colon have similar pattern of contractions and relaxation (Figure 3). The relaxation takes one half of the process and the first and the second part of the contraction are with almost identical proportion, The other parts of GIT - stomach, duodenum and rectum have almost similar pattern of contractions and relaxations. Only ilium differs from these two groups. The relative part of its relaxation was 0.75 from whole duration of process. Application of the time parameters clearly shows the presence of bilateral symmetry in the responses of the gastrointestinal tract.

The amplitude comparison of the Ang II-induced contractions divides the isolated smooth muscle preparations into two groups. The stomach and the small intestines form one group, and the large intestines form another. It is obvious that the large intestines are more sensitive to Ang II and react with more powerful contractions. There is a gradual increase in

Figure 3. Normalized time-parameters of contractile activity of different GIT segments, induced by Angiotensin II. 1-T_{hc}, 2-T_{(c-hc)n} and T_{hm}.

the muscle response to Ang II along the rat intestine, which confirms previous studies of Ang II - provoked intestinal contractions (Ewert et al., 2006). In the literature there are evidences about the uneven distribution of the Ang II receptors in most tissues of the adult organism (Steckelings et al., 2010). Regarding GIT there has been described an unequal density of AT1 receptors (Fändriks, 2010), which could explain the obtained results. From the other side, the duodenal contraction has the smallest amplitude, which strongly differentiates the reaction of the duodenum from the other GIT segments. This could be due to low density of duodenal Ang II receptors and with a local production of NO by the duodenal mucosa (Aihara et al., 2005).

There had been established several transduction pathways of Ang II- induced SMC (Dinh et al., 2001; Romero et al., 1998). The modulating effect of Ang II on different ion currents also had been reported (Chorvatova et al., 1996; Romero et al., 1998). According to T_{hc} and T_c parameters, there is a marked difference between the stomach from the one side, and the intestines from the other. All of the studied intestinal segments showed similar speed of contraction, while the duration of the stomach reaction was far longer. That data suggest a possible transduction pathway for SMC of the stomach, different than in the others GIT segments.

Some authors consider possible competitive interactions between AT1 and AT2 receptors in smooth musculature of the intestine, which supports some previous statements that the

magnitude of the response to Ang II depends on the expression of both receptors (Ewert et al., 2006). It had been demonstrated that only AT1 receptors are relevant for the maximum response in ANG-induced contractions (Hawcock & Barnes, 1993; Fändriks, 2010; Fan et al., 2002; Rattan et al., 2003; Ewert et al., 2006; Fändriks, 2010). Despite the existing assumptions that stimulation of AT2 receptors may have the opposite effect than that of AT1 (Gallinat et al., 2000), the role of AT2 receptors for the relaxation phase of the SMC in GIT is not examined. The importance of AT2 receptors for the relaxation of the rectum has been described only (De Godoy et al., 2006; De Godoy & Rattan, 2005). Regarding the time parameters for relaxation, the stomach again showed the slowest response. In this case the ileum indicated significantly prolonged reaction compared to the other intestinal segments. The reason for that difference may be the complete absence or the low density of AT 2 receptors in the ileum (Fändriks, 2010).

In conclusions the observed differences in the Ang II - induced gastro-intestinal contractions may be due to:

- Variation in the Ang II receptor subtypes distribution. Regarding GIT there has been described an unequal density of Ang II receptors. This uneven distribution of the receptors could explain the differences in the amplitude and duration of T_{hc} of SMC.
- Counteraction between Ang II receptor subtypes. Competing actions between Ang II receptors have been discussed in the smooth musculature of rat small intestine. The relative receptor expression is a determinant of the magnitude of response to Ang II. This might be of importance for the duration of muscle contraction after reaching the maximal response - expressed by T_{hr} .
- Activation of various transduction pathways. There is data that Ang II can modulate ionic conductance in distinct tissues. The different duration of the interval between T_{hc} and T_c , as well as T_{hr} may be due to the involvement of some membrane ion channels.
- Presence of local rennin - angiotensin system and formation of numerous of active angiotensin derivates. It is proven that most tissues are the source, target and degradation site of Ang II. Furthermore, local rennin - angiotensin system or parts of it had been found in rat rectum and rat small intestine. This is another possibility which could explain the obtained data about the phase of relaxation and force of SMC.
- The use of time - parameters significantly contributes to the analysis of the contraction process and permits a good comparison of the Ang II – induced responses. Presentation of the time parameters as part of the total contraction normalization) gives an idea for the development of the process in the different time intervals.
- The obtained results provide a direction for further research work on Ang II-mediated contractions of GIT and for clarifying the exact role of the AT1 and AT2 receptors in the different phases of SMC.

Ang II – provoked rectal response. Comparison with the urinary bladder response

The application of Ang II on the rectal preparation caused a development of expressed tonic contraction, which amplitude and integral muscle force were significantly greater than those of the bladder. The higher amplitude is achieved at the expense of the second half of the

contraction. The higher values of the absolute and normalized time – parameters for this interval are the evidence. The difference in the total muscle mass of the preparations significantly contributes for these distinctive force parameters. It is worth noted, that the time-parameters (absolute and normalized) of Ang II – mediated bladder and rectal SMC, with the exception of $T_{(c-hc)}$ parameter, do not indicate significant differences (Figure 4). This proves the suggestion that in the urinary bladder and rectum the Ang II - mediated contractions are developed by similar mechanisms. Moreover, this assumption is an indirect evidence for an approximately equal density of Ang II receptors in these two organs. The uniformity of response to Ang II is supported by the fact that in the rectum a local renin-angiotensin system has also been established (De Godoy & Rattan, 2006). It could be considered again that the locally generated metabolites of Ang II contribute for this pattern of the contraction process.

Figure 4. Normalized time-parameters of urinary bladder and rectum Ang II – induced contractions. All of the normalized time-intervals were calculated as a relative part from Tchr.

Does AVP have an importance for the motility of the gastro-intestinal tract?

Dose-dependent effects of AVP on gastro-intestinal tract from different species were observed, but regarding the rectal musculature the information is insufficient and

controversial (Ohlsson et al., 2006). AVP has been shown to increase the gastric and duodenal motility in humans and rabbits (Ohlsson et al., 2006; Li et al., 2007), as well as colonic peristalsis, but the expression of the AVP receptors in intestine has not been examined yet (Ohlsson et al., 2006). Some authors have demonstrated that AVP increase the gastro-intestinal motility via the oxytocin OT1 receptors, but the experiment is only for stomach and duodenum from rabbits (Li et al., 2007).

In our study, the application of AVP does not significantly alter the characteristics of the spontaneous phasic contractile activity of gastro-intestinal segments except this of stomach. This could be explained with the absence of AVP receptors type V1, which are present in the urinary bladder. In rectal musculature V2 receptors could be presented – in such a case, the rectum as a terminal department of gastro-intestinal tract shows analogy with the distal and the collecting tubules of the kidneys. This is still an assumption that remains to be investigated.

Gallbladder

The mechanical activity of the gallbladder of conscious dogs consisted of spontaneous rhythmic contractions with a frequency of 2 to 5 cpm. Fluctuations of the tone were also observed. Bolus injection of cholecystokinin octapeptide i.v. produced a dose-dependent increase in gallbladder pressure (Figure 5A). Atropine decreased gallbladder pressure and reduced or even abolished the cholecystokinin action (Figure 5B). Hexamethonium led to gallbladder relaxation and also greatly reduced the gallbladder response to cholecystokinin octapeptide (Figure 5C).

The mechanisms through which atropine or hexamethonium inhibit cholecystokinin-produced gallbladder contractions under in vivo conditions are not understood. One possible explanation might be that the excitatory effect of cholecystokinin on the gallbladder is mediated by acetylcholine release from cholinergic neurons at pre- and post- ganglionic level. Another possibility is that atropine and/or hexamethonium are able to block the release of endogenous cholecystokinin from the endocrine cells or neurons. This suggestion is supported by the fact that vagotomy abolish gallbladder response to cholecystokinin after acidification (Fried et al., 1983), infusion of fat into the duodenum (Magee et al., 1984) or after drinking water (Sundler et al., 1977). The release of CCK in the circulation in response to fat or other meals is also reduced after vagotomy or atropine. It is also possible that atropine or hexamethonium blockade of cholinergic input to the gallbladder may unmask the release of neuronal inhibitory influence which could then compete with the release of CCK. Such inhibitory agents could be somatostatin and vasoactive intestinal peptide (Lenz et al., 1993; Milenov et al., 1995).

Uterus

It has been reported that in the uteri of a number of species, local production of Ang II and the enzymes for its synthesis are present. Besides the proven contractile effect of Ang II on the uterine arteries, research in this area showed that myometrium is also sensitive to the effect of this octapeptide (Keskil et al., 1999).

Figure 5. CCK-8 (2.5; 5 and 10 ng/kg i.v.)-induced gallbladder pressure before (A) and after atropine (B) (20 μ g/kg i.v.) or hexamethonium (C) (1 mg/kg i.v.). Means $\pm$ S.E.M. of 12 experiments in 6 conscious dogs are presented, $P < 0.01$.

There is substantial evidence for the involvement of AVP in conditions of uterine hyperactivity. Even more, it has been shown that the human myometrium is more sensitive to AVP than to oxytocin (Bossmar et al., 2007).

Ang II at concentration of 1 μ mol induced tonic contraction with maximum amplitude of 6.00 ± 0.22 g and an integral force of muscle contraction of 1150.00 ± 614.70 gs. AVP applied

in the same concentration as Ang II induced tonic contractions with amplitude of 6.61 ± 0.39 g ($n = 8$) and an integral force of muscle contraction of 7245.00 ± 901.00 gs. The duration of the AVP-induced responses was several times greater than those of Ang II and the recording of AVP-mediated contractions was stopped on the 30th minute without achievement of T_{chr} parameter.

Our experiment confirmed the contractile effect of these two peptides on the myometrium, which is in accordance with the results of other authors working on the same issues (Anouar et al., 1996; Chan et al., 1996; Keskil et al., 1999).

The contractions induced by both peptides have similar amplitudes, but they are with different duration and characteristics. The registered AVP - provoked uterine responses were found to have a sustained oscillating character (Figure 6). When analyzed by mathematical modeling such contractions were recognized as underdamped process - the system tries to establish a stable level different from the baseline (Yankov, 2009). The differences in the developed contractions may be due to split of the classical or inclusion of additional transduction pathway for each of the studied peptides. Both of them have several main groups of receptors. The receptors for Ang II are AT1 and AT2 (De Gasparo et al., 2000), while the receptors for AVP are V1a, V1b and V2 (Petersen, 2006).

Figure 6. Original record of vasopressin-induced uterine contraction.

To establish the importance of these receptors for the uterine muscle contraction will be the subject of our next experiments. However, several interesting facts immerge:

First – the constrictor effect of Ang II is associated with AT1 receptors, but the uterus is one of the few organs with a. uterina inferior where AT2 receptors are predominant (Keskil et al., 1999). AT2 receptors are mainly regarded to oppose the effects of AT1 and cause dilation, blood pressure reduction, nitric oxide production (Hannan et al., 2003). Perhaps the significantly shorter phase of contraction and relaxation was due to their activation under the influence of Ang II in the uterus. Second – the constrictor effect of AVP is realized by V1a receptors which are found in uterine arteries. With regard to the contractile response of the myometrium, however, there are statements that the resulting contraction from the AVP influence is due to activation of other receptors, different from the mentioned above (Anouar et al., 1996). Some authors go even further and argue that AVP accomplish its effect on uterine musculature by OT receptors, which have big similarity with V1 receptors (Chan et al., 1996).

Considering that both peptides are released from supraoptic nuclei in the hypothalamus and that they have a powerful contractile effect on the smooth muscle, it is appropriate to

search a closer connection between them in preparing the uterus for pregnancy and labor. Probably these two peptides act synchronously which potentiate their own effects (Douglas et al., 2001).

Studies on rats show that AVP is more potent uterotonic agent than OT in non pregnant condition (Bossmar et al., 2007) and during parturition OT predominantly promotes uterine contractions, while AVP is more important for vasoconstriction, thus reducing the bleeding after delivery (Chan et al., 1996; Douglas et al., 2001).

The study of Ang II – and AVP – mediated uterine contractions contributes considerably for the revealing of the mechanisms that generate and modulate uterine activity. This could be beneficial for a better understanding and control of myometrial dysfunction.

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