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EFFECTS OF SOME VASOACTIVE NEUROPEPTIDES ON MOTOR ACTIVITY OF SMOOTH MUSCLE ORGAN´S STRIPS FROM DIFFERENT AREAS OF GASTROINTESTINAL SYSTEM

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Abstract

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The aim of this study was to analyze in detail and to compare the effects of Angiotensin II (Ang II) and Arginine - vasopressin (AVP) on the contractile activity of smooth muscle strips from different rat gastrointestinal segments by application of time-parameter analysis. Longitudinal muscle strips from the rat stomach and intestine were used for in vitro recording of contraction, iduced by Ang II (10^{-6} M) and AVP (10^{-6} M). Amplitude, area under the curve (AUC) and time-parameters of the curves force-vs.-time of the contraction were determined. The colon and rectum responded to Ang II with more powerful contractions (3.43 ± 0.56 g and 4.74 ± 0.65 g, respectively). Jejunum and colon from one side and stomach, duodenum and rectum on other hand showed a similar pattern of contractions and relaxations. The response of the ileum was different. It was shown bilateral symmetry in the responses of the gastrointestinal tract. The differences in the responses of smooth muscle strips on Ang II in the various segments are probably due to unequal distribution of the density and opposite effects of AT_1 and AT_2-receptors, the presence of local RAS and activation of various transduction pathways. AVP induced tonic contractions of the preparations from the stomach. In the intestines, AVP was ineffective.

Key words: smooth muscle strips, organ baths, angiotensin II, vasopressin

Abbreviations: ACE – Angiotensin-converting enzyme; Ang II – Angiotensin II; AUC – Area under the curve; AVP – Arginine-vasopressin; DAG – Diacylglycerol; GIT – Gastro-intestinal tract; IP_3 – Inositol triphosphate; RAS – Renin-angiotensin system; SMC – Smooth muscle contractions

Introduction

It is well known that the renin-angiotensin system (RAS) is a prominent regulator of blood pressure, blood volume, body fluid, electrolyte balance and the sympathetic nervous system (Dinh et al., 2001). With regard to renal, cardiovascular and and central nervous systems, the RAS has been a subject of extensive research. However, few studies have been devoted to the impact of the RAS on the alimentary system, although it is fundamental for the intake and excretion of fluid and electrolytes, and for the bodily haemodynamics.

The main effector of the RAS - Ang II, is a highly active octapeptide that is generated in the circulation or locally in tissues of the kidney, blood vessels, heart, brain and etc. Ang II may thereby mediate autocrine, paracrine and intracrine effects. Today, we know that the requisite components of the RAS, as renin, angiotensinogen and angiotensin-converting enzyme (ACE) are present in such tissues and also can be locally well expressed and active in the gastrointestinal tract (GIT). Furthermore, Ang II can be formed via non-ACE and non-renin enzymes including chymase and cathepsin G (Dinh et al., 2001). Local RAS or parts of it had been found in rat rectum (De Godoy and Rattan, 2006), small intestine and other (Leung et al., 1993). The role of Ang II was confirmed in the development of diseases such as gastro-esophageal re-

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flux, Crohn's disease (Fändriks, 2010), incontinence of internal anal sphincter (De Godoy and Rattan, 2006; Rattan et al., 2003) and others.

Ang II has been implicated in a wide range of physiological processes by the activation of two different subtypes of G protein-coupled receptors, AT_1 and AT_2. For many years, most of the effects of Ang II were attributed to the activation of the AT_1 receptors, but recently more often is discussed the involvement of AT_2 receptors in the actions of Ang II (De Godoy and Rattan, 2006; Fändriks, 2010; Chorvatova et al., 1996; Romero et al.,1998).

There are several reports showing Ang II regulation of intestinal fluid and electrolyte transport. A small number of pharmacological studies has established the role for Ang II as a potent endogenous activator of gastrointestinal smooth muscle motor activity, but its actions on gastrointestinal wall musculature have not been thoroughly investigated. A number of studies has shown the presence and actions of the Ang II receptor subtypes at various locations along the GIT. The presence of its receptors in the tissue can be used as an indicator of potential physiological actions. For example, a novel data suggest that the AT_1 receptor mediates muscular contractions, that the AT_2 receptor regulates epithelial functions, and that the AT_2 receptors counteract effects mediated by AT_1 receptors in various tissues (Fändriks, 2010). There is evidence that AT_1 receptors are situated directly on the smooth musculature (Romero et al., 1998) and AT_2 receptors are not found in direct association with the smooth muscle cells (Schinke et al., 1991). The signal transduction mechanism for AT_1 receptors is well known. These receptors activate phospholipases A_2, C, D and L-type Ca^{2+} channels and inhibiting the adenylyl cyclase (Shokei and Hiroshi, 2011). In addition, in some cells, stimulation of AT_1 receptors induces a rapid phosphorylation of several tyrosine residues including PLC-y1 and the intracellular kinases Jak2, inducing the activation of Jak-STAT signal transduction (Chorvatova et al., 1996). There is evidence that in various cell lines, AT_2 receptors activated protein tyrosine phosphatase. Another target of AT_2 receptors are the membrane currents (Chorvatova et al., 1996; Kim and Awao, 2011).

AVP is a nonapeptide that is synthesized in neurons located in the paraventricular and supraoptic nuclei of the hypothalamus and is released into the bloodstream in the posterior pituitary (Swaab et al., 1975).

The physiological effects of vasopressin are concerned with the regulation of water metabolism, osmolality of body fluids and blood pressure. The effects of AVP are mediated mainly via V_1 and V_2 receptors. V_1 receptors are located on the smooth muscle cell membranes in blood vessels, urinary bladder and myometrium. V_1-receptor activation mediates vasoconstriction by receptor-coupled activation of phospholipase C and release of Ca^{2+} from intracellular stores via the phosphoinositide cascade (Briley et al., 1994). V_2 receptors are present in the renal collecting tubules and endothelial cells. V_2 receptors of the kidney increase the intracellular cAMP, interacting with adenyl cyclase and causing water retention (Orloff and Handler, 1967). The effects of AVP on GIT from different species are observed, but the information is controversial and insufficient.

The question for the exact effects of Ang II and AVP on smooth muscle contractions (SMC) of GIT remains still opened. There is not enough information in the literature, regarding the comparative characteristics of Ang II and AVP-induced responses from the various segments of alimentary tract. Dose-dependent curves are commonly used as a method for studying of SMC provoked by different agents (Leung et al., 1993; Hawcock and Barnes, 1993; Park et al., 1973). Dose-response curves may give information on effective doses and for the maximum responses to applied substances (Fändriks, 2010). In the literature, there is no data for other important characteristics of SMC, provoked by Ang II and AVP. The length of the phases of contraction in the different gastrointestinal segments is not clarified and analyzed by application of a time-parameter analysis. Such studies were performed on the skeletal muscle contraction (Raikova and Aladjov, 2004).

Thus, research aims to study in details and compare the effect of Ang II and AVP on contractile activity of smooth muscle strips from different departments of rat alimentary tract by using time - parameters of the different phases of the SMC.

Materials and Methods

In vitro experiments were performed on male and female Wistar rats weighing 200–250 g. The experimental protocol of the study was approved by the Institutional Animal Care and in accordance with the national regulations, and European Directive of 22.09.2010 (210/63/EU) concerning the protection of animals used for scientific and experimental purposes. The animals were anesthetized with Nembutal 50 mg/kg intraperitoneally and exsanguinated.

Tissue preparation and recording of the SMC

For *in vitro* recording of Ang II and AVP-induced contraction, smooth muscle preparations of stomach fundus (n=10), duodenum (n = 6), jejunum (n = 6), ileum (n = 11), colon (n = 8) and rectum (n = 7) were used. Abdominal cavity of the rats was opened and segments from different parts of intestines with a length of 8 mm were dissected out. Longitudinal

smooth muscle fundic strips (2 mm wide, 0.5 mm thick and 8 mm long) was dissected from the area of greatest curvature in the stomach. The isolated organs were transferred immediately in cold Krebs' physiological solution (3°C) containing (in mM): NaCl-118.0, KCl-4.74, $NaHCO_3$-25.0, $MgSO_4$-1.2 $CaCl_2$-2.0, KH_2PO_4-1.2 and glucose-11.0. The two ends of each segment or 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 (model FSG-01 Experimetria, Ltd., Hungary). The preparations were mounted in organ baths (model TSZ-04/01; 10 ml chamber volume), containing oxygenated with Carbogen (95%O_2 and 5% CO_2) Krebs'solution at pH 7.4 and temperature 37°C. The smooth muscle strips were initially stretched under 1.0g resting tension followed by 90 minutes of equilibration. During equilibration, the bathing fluid was replaced with fresh Krebs'solution at 15-th min, 60-th min and 75-th min. After the initial period of adaptation, the phasic contractions of the smooth muscles before application of Ang II and AVP were registered, and the preparations were treated with the solutions of Ang II and AVP in a dose of 1μmol ($1x10^{-6}$M), added to the 5 ml of Krebs' solution in the organ baths. Single large dose of Ang II and AVP were applied, in the experiments, in order to measure the maximum effect. Ang II and AVP were added into the baths noncumulatively. Ten minutes after the applications of neuropeptides, the baths were washed out.

The changes of motor activity, expressed as tonic contractions, relaxations or lack of reaction after treatment with Ang II and AVP were displayed and recorded. The mechanical acyivity was transformed by mechanical-force sensor, amplified, digitized and recorded using digital acquisition software ISOSYS-ADVANCED 1.0, produced by Experimetria Ltd., Hungary.

Drugs and compounds used

Angiotensin II, Arginine - vasopressin and all reagents for the preparation of Krebs' solution were purchased from Sigma-Aldrich (St. Louis, MO).

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 were performed with KORELIA–Processing software (Yankov, 2010).

Following time-parameters were examined (Yankov, 2011): T_{hc} , $T_{(c-hc)}$, T_c , T_{hr} , T_{chr} (Figure 1):

T_{hc} (s) – first half contraction time: time interval between the start of the 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} ($T_{chr} = t_{hr} - t_0$).

The duration of interval for analysis of the tonic contraction induced by Ang II or by AVP was defined from the beginning of the contraction, until the amplitude fell to 50%.

When the responses to Ang II were investigated, the different intervals were normalized as a relative part of total length of the process ($T_{xn} = T_x/T_{chr}$) for a more correct and acurate comparison between the parts of the gastrointestinal tract. As a result the following normalized parameters were obtained: $T_{hc}n$, T_cn, $T_{(c-hc)}n$, $T_{hr}n$.

Statistical analysis was performed with the statistical program Statistica 8.0, StaSoft, Inc. The results are presented as mean ± standart error. A p-value ≤ 0.05 was considered statsitically significant.

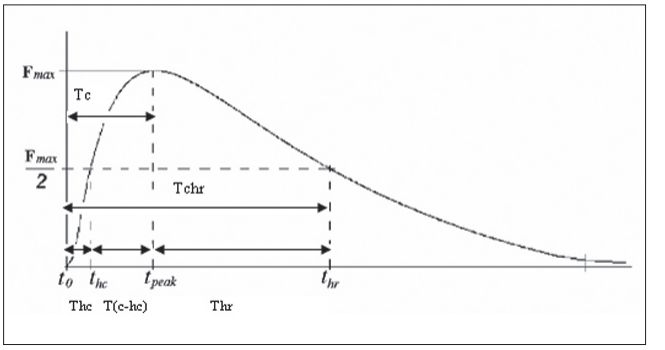

Fig. 1. Smooth muscle contraction (SMC) - graph and parameters

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 $F_{max}/2$; T_{hc} – half contraction time: time interval between the start of the SMC and $F_{max}/2$; $T_{(c-hc)}$ – second half-contraction time: time interval between $F_{max}/2$ and F_{max}; T_c – contraction time: time interval between the start of the SMC and F_{max}; T_{hr} – half-relaxation time: time interval between F_{max} and $F_{max}/2$; T_{chr} – contraction plus half-relaxation time: time between the start of the SMC and $F_{max}/2$

Results

Effect of Ang II on the amplitudes and integral muscle force of SMC

Our experiments show that the amplitudes of angiotensin II-induced contractions of smooth muscle of different segments from digestive tract are similar in the stomach (1.14 ± 0.13 g), jejunum (1.11 ± 0.14 g) and ileum (1.09 ± 0.16 g) (Figure 2). These values do not differ statistically significantly from each other ($P > 0.05$, t-test). The smooth muscle strips of the duodenum showed the lowest amplitude of contraction (0.55 ± 0.13 g) in the small intestine. Under the influence of Ang II, amplitude of contractions of segments of the large intestine is greater than that of the stomach and small intestine and the amplitude of rectum (4.74 ± 0.65 g) is greater than that of the colon (3.43 ± 0.56 g). However, the integral force of stomach contraction (178.09 ± 19.63 gs) is significantly greater ($p < 0.05$) than that of the duodenum (41.43 ± 15.52 gs), jejunum (92.33 ± 8.01 gs) and ileum (100.75 ± 14.07 gs) and is equally powerful ($p > 0.05$) as that of the colon (162.25 ± 26.60 gs). The segments from the duodenum showed again the smallest integral force of contraction and these from the rectum developed the most powerful integral force (328.43 ± 75.23 gs).

The amplitudes and integral muscle force of the different segments from GIT in this experimental study showed marked correlation one another ($r = 0.88$; $P < 0.05$).

Analysis of the time parameters of Ang II-induced contractions

The analysis of time - parameters of the contractions indicated that the gastric response to Ang II required more time to develop (Figure 3). The time intervals T_{hc} (29.09 ± 2.53 s) and T_c (78.18 ± 5.87 s) of the stomach contraction are more extended compared with T_{hc} and T_c parameters of the registered intestinal contractions.

This tendency for a slower progress of the reaction is maintained during the whole contraction of the stomach muscle strips. Time interval T_{hr} (146.73 ± 15.17 s) from the relaxation of the stomach strips and time interval T_{chr} (224.90 ± 18.45 s), which represents the overall reaction of the stomach on Ang II, are prolonged in comparison to the same time - parameters of the intestinal contractions. The results from the intestinal contractions in respect of the time parameters T_{hr} and T_{chr} are analogous. Exceptions are only the time - parameters T_{hr} (106.33 ± 9.89 s) and T_{chr} (141.08 ± 9.48 s) of the ileum, which are significantly extended. It is noticeable that the responses of the colon and rectum are characterized by approximately equal time - intervals for contraction and relaxation.

Analysis of the normalized time parameters of Ang II-induced contractions

After normalization of the time - parameters, it is noteworthy that jejunum and colon have identical pattern of contractions and relaxation (Figure 4). The phases of the contraction (T_{hc} n and $T_{(c-hc)}$ n) are in almost equal proportions and the relaxation includes half of the duration of the process. Regarding the normalized time – parameters, similar pattern of reaction is observed also in the preparation from the stomach, duodenum and rectum. It is notable that the duration of the relaxation of ileum is 0.75 from whole contraction process. After the application of the normalized time – parameters, the presence of bilateral symmetry in the responses of the digestive tract can be clearly seen.

Effect of AVP on the amplitudes and integral muscle force of SMC

In our study, the application of AVP does not significantly alter the characteristics of the spontaneous phasic contractile activity of intestinal segments ($P > 0.05$; t-test). Only smooth

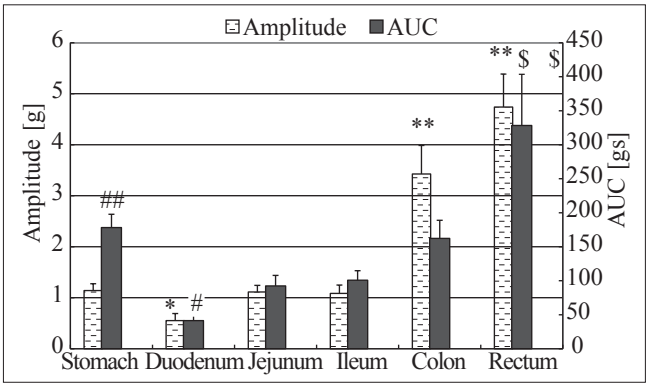

Fig. 2. Amplitudes and integral forces (AUC) of smooth muscle preparations from different rat gastrintestinal segments under influence of Ang II

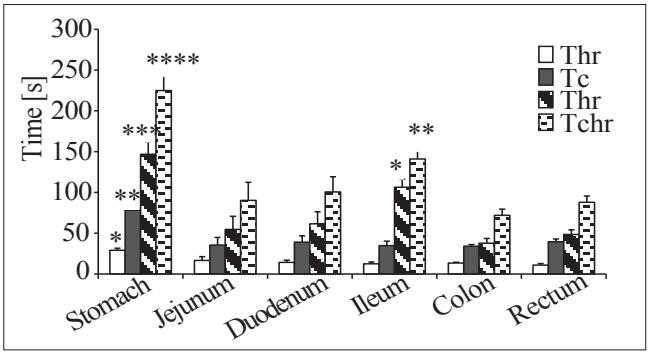

Fig. 3. Time parameters of the induced by Ang II tonic contractions of some gastrointestinal segments

T_{hc} – half contraction time; T_c – contraction time; T_{hr} – half-relaxation time; T_{chr} – contraction plus half-relaxation time

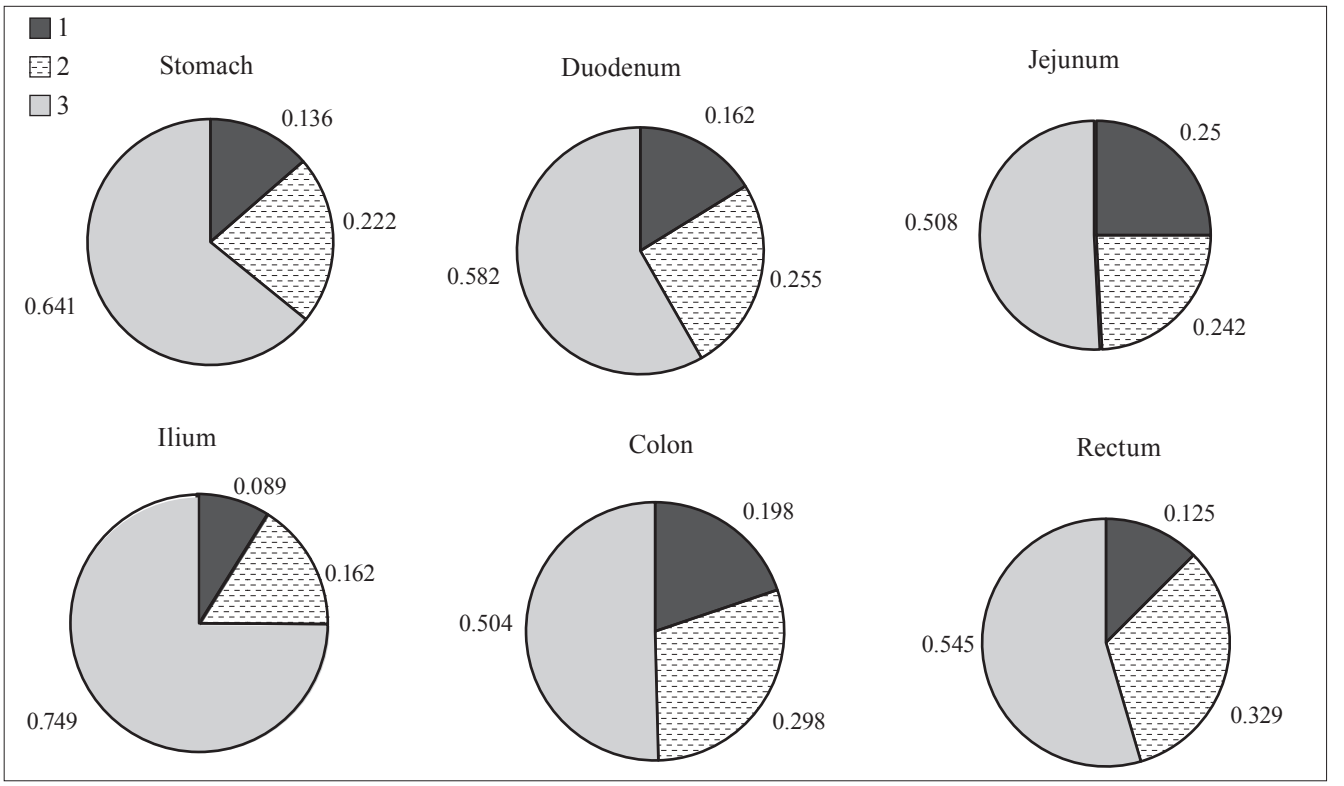

Fig. 4. Normalized time parameters of contractile activity of different gastro-intestinal segments, induced by Ang II 1-T_{hc}n, 2-$T_{(c-hc)}$n and T_{hr}n. All of the normalized time intervals were calculated as a relative part from T_{chr}

muscle strips from the stomach developed a tonic contraction with amplitude 2.73 ± 0.26 g and integral force 1618.20 ± 273.26 gs which are significantly higher compared with the Ang II-induced stomach contraction.The relaxation was very slow, but the contraction was faster than that one, induced by Ang II.

Discussion

The conducted experiments with isolated tissues confirmed the potent contractile effect of Ang II on the smooth muscles of GIT. There had been established several paths for signal transduction of Ang II-induced SMC. The most-studied signal transduction by Ang II is its binding to the AT_1 receptor, leading to the formation of two second messengers, inositol trisphosphate (IP_3) and diacylglycerol (DAG). The IP_3 causes an increase of the cytosolic Ca^{2+} concentration due to the opening of IP_3-sensitive Ca^{2+} channels from the sarcoplasmic reticulum, thus triggering contraction. In addition, DAG activates protein kinase C, which may in turn stimulate protein phosphorylation and ionic channel activation (Romero et al.,1998). It is known that Ang II modulates various ionic currents and these effects provoke changes in membrane potential, such as modulation of action potential firing or resting membrane potential and control intracellular Ca^{2+} concentration (Chorvatova et al., 1996). For example, Ang II may induce an increase of Ca^{2+} currents, blockade of K^+ channels, and depolarization of the cell. Furthermore, in the guinea pig ileum, Ang II depolarizes the cells by activating nonselective cation channels and/or voltage-gated Ca^{2+} channels, leading to enhanced Ca^{2+} and Na^+ influxes. In many smooth muscles are described Ca^{2+} -dependent K^+ channels (maxi-K^+ channels) that are activated by depolarization. Other ionic currents, such as chloride currents, are also altered by this neuropeptide (Chorvatova et al., 1996; Romero et al., 1998).

The data for the gradual increase of the amplitude and integral force of the smooth muscle contractions along the rat intestine, which we received in our experiments, confirm previous studies of Ewert et al. (2006) on Ang II-induced intestinal smooth muscle contractions in rats.

In our experiments are formed two groups of isolated smooth muscle preparations, which differ from each other in the size of the amplitude of its Ang II-induced contractions. The first group includes the stomach and small intestines. The second group covers the sections of the large intestines: colon

and rectum. It is obvious that the sensitivity of the large intestines on Ang II is bigger and they react with more powerful contraction compared with the rest of the digestive tract. Obviously, Ang II has greater importance for these segments in the performance of their evacuator function. In support of this assumption, there is a literature data about the uneven distribution of the Ang II receptors in most tissues of the adult organism (Steckelings et al., 2010). The density of AT_1 receptors along the digestive tract is also not uniform (Fändriks, 2010).

The registrated lowest amplitude of the duodenal contractions, compared to other parts of the digestive tract, is probably due to the low density of duodenal Ang II receptors but also probably due to the action of common path for local production of bradykinin, NO and prostaglandins by the duodenal mucosa (Aihara et al., 2005; Ewert et al., 2003). Activation of phospholipases A_2 and D stimulates the release of arachidonic acid, the precursor molecule for the generation of prostaglandins (Dinh et al., 2001). The described AT_2 receptors in the digestive tract (De Godoy and Rattan, 2006; Leung et al., 1993; Fändriks, 2010) are associated with the exchange of water and salts, secretion of sodium hydrogen carbonate in the duodenum (Fändriks, 2010) and secretion of nitric oxide in the jejunum (Ewert et al., 2003). For AT_2 receptors is known that they also use different signal transduction pathways, such as activation of various phosphatases, cGMP-NO system, etc. (Dinh et al., 2001; Ewert et al., 2003), but their actual signal transduction is not quite elucidated.

Regarding the measured parameters – T_{hc} and T_c, there is a considerable difference between the response of the stomach from one side and the intestine from other hand. Under the influence of Ang II the speed of contraction of the stomach is small, while the speed of the contractions in the intestine is higher and its values in all investigated intestinal segments are similar. According to these data it may be suggested that the signal transduction mechanism of the stomach SMC is different compared to other intestinal segments.

The results from the comparison of time parameters of the relaxation displayed again that the stomach has the slowest response. Ileum also showed significantly longer reaction compared to the other intestinal segments. The reason for this difference may be the low density of the AT_2 receptors in the ileum or their complete absence (Fändriks, 2010). The importance of the AT_2 receptors for motor activity of the digestive tract is still in a process of clarification. Gallinat et al. (2000) assumed that they have the opposite effects of AT_1 the receptors, but their importance for the smooth muscle relaxation has been demonstrated only for the internal anal sphincter (De Godoy and Rattan, 2005; De Godoy and Rattan, 2006a). According to Ewert et al. (2006), there are possible competitive interactions between AT_1 and AT_2 receptors in smooth muscle of the intestines, which supports some previous statements that the magnitude of the response to Ang II depends on the expression of the two receptors. An internalisation of AT_1 receptors and externalisation of AT_2 receptors may be responsible for the activation of the AT_2 receptors, which leads to the relaxation at higher concentrations of Ang II (Fändriks, 2010; De Godoy and Rattan, 2005).

Does AVP have an importance for the motility of the gastrointestinal tract?

There is evidence that AVP exert dose-dependent effects on the digestive tract in different species. For example, it is proven that AVP increases the motility of the stomach, duodenum and colon in humans and rabbits, but information about its effects on the smooth muscles of the rectum is controversial. Expression of the AVP receptors in intestine has not been examined yet (Li et al., 2007; Ohlsson et al., 2006). Li et al. (2007) considered that AVP might increase the motility of the stomach and duodenum using oxytocin receptors OT_1.

In our study, the application of AVP did not significantly alter the characteristics of the spontaneous phasic contractile activity of the various gastrointestinal segments, with exception of the stomach. This could be explained with the absence of AVP receptors type V_1 in the intestine of the rats.

Conclusions

In summary, the present experiments indicate the existence of bilateral symmetry of the gastrointestinal responses to the effects of Ang II. The observed variations in the characteristics of Ang II-induced gastrointestinal contractions may be due to the following reasons: 1) Fluctuations in the distribution and unequal density of the Ang II receptor subtypes in the various segments of GIT, which may explain the differences in the amplitude and the duration of T_{hc} of SMC. 2) Opposing effects of the Ang II receptor subtypes in the smooth muscle of the small intestine.

The relative expression of the Ang II receptors is a factor that determines the response to Ang II. This might be of importance for the duration of muscle contraction, after reaching the maximum response to Ang II - presented by T_{hr}. 3) Activation of different signal transduction pathways and modulating effect of Ang II on the conductance of some ion channels, which determines the different duration of the interval between T_{hc} and T_c, as well as T_{hr}. 4) Existence of local RAS and formation of active angiotensin derivatives.

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 (nor-

malization) 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 alimentary tract and for clarifying the exact role of the AT_1 and AT_2 receptors in the different phases of SMC.

Acknowledgments

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