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Angiotensin II and Vasopressin effects on motor activity of rat isolated tissue strips from urinary bladder and rectum

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The purpose of this study was to analyze and compare the force and time-parameters of Angiotensin II (Ang II) and Arginine-Vasopressin (AVP)-provoked contractions on muscle strips from rat urinary bladder and rectum in experiments in vitro. Mature Wistar rats, weighting 250–300g, were used. Longitudinal strips from urinary bladder and rectum were prepared and influenced by Ang II and AVP in a dose of 10^{-6} M. The recorded force-vs.-time curves were analyzed including calculation of amplitudes, area under the curve (AUC) of the smooth muscle contraction, as well as defining of different time-parameters. Ang II and AVP caused urinary bladder tonic contractions with similar amplitudes (1.74 ± 0.27 g and 1.55 ± 0.16 g, respectively) and different AUC. Marked difference was observed in the application of both peptides on strips from rectum. Ang II caused tonic reactions with amplitude of 4.60 ± 0.42 g, while AVP do not change significantly phasic contractions. The time-parameters analysis established an analogy in the developed response to Ang II of both organs.In urinary bladder, the action of Ang II derivatives and the interactions of the two peptides with the ion channels of the plasmalemma might be the reason for the observed differences in the contraction parameters.The similarity in the time-parameters of Ang II-mediated contractions of the bladder and the rectum indicates an analogical mechanism of the development of the contraction. The lack of a rectal tonic response when AVP was applied is probably due to different type of the receptors or modifications in the transductional signal pathway.

Key words: Angiotensin II, Vasopressin, rectum, urinary bladder, time-parameters

INTRODUCTION

The growing incidence of micturition disorders and faecal incontinence focuses attention of many researchers on the study of motor activity of the urinary bladder and the recto-anal segment of gastro-intestinal tract. These two organs have mainly a reservoir and evaquatory functions. The maintenance of their adequate tone is essential for a normal quality of life. The precisely coordinated and complex smooth muscle activity of bladder and rectum is regulated by interplay between neural (somatic and autonomic) and endocrine control mechanisms.

The neuropeptides Angiotensin II (Ang II) and Arginine-Vasopressin (AVP) are important factors in the regulation of the blood vessels tone. Furthermore, there is growing evidence for the involvement of these two peptides in the regulation of the smooth muscle activity outside the vascular system, such as urinary bladder and distal segments of the gastrointestinal tract [1–5].

The physiological role of Ang II for the function of the urinary bladder and the exact mechanism mediating its effects has not been fully revealed. According to experimental data of Anderson and co-workers [6], Ang II has a possible role in the micturition. It is proven that Ang II and its precursor Ang I cause dose-dependent contractions of muscle strips from rat urinary bladder [7], and probably act as modulators in neurotransmission in this organ [1]. There are evidences that Ang II accomplish its physiological effects by binding to AT1 receptors [8], whose number in the membrane of the detrusor smooth muscle cells can vary significantly [9, 10]. AT1 receptors activate phospholipase C (PLC), dihydropyridine-sensitive Ca^{2+}-channels and inhibit adenylilcyclase, reducing intracellular cAMP [11].

Except the well-known constriction of the vessels, AVP affects the contractility of the urogenital smooth muscle, manifested in experiments with rats [1]. It is proven the presence of V1-receptors in smooth muscle of the urinary bladder, whose binding to AVP also leads to activation of the IP_3 pathway, similarly to binding

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of Ang II to AT1 [12, 13].

There is not enough information in the literature, regarding the exact effects of Ang II and AVP on the recto-anal segment of GIT. Local renin-angiotensin system or parts of it had been found in rat rectum [14]. The role of Ang II had been confirmed in the development of diseases such as internal anal sphincter incontinence [4, 5]. In recent years the significance of AVP as an important regulator of the gastro-intestinal smooth muscle activity is growing [15, 16]. Regarding the tone of the rectal musculature this question remains still opened.

The purpose of this study was to analyze and compare in details the registered Ang II and AVP-provoked contractions in experiments in vitro on muscle strips from rat urinary bladder and rectum.

EXPERIMENTAL METHODS

Sample Preparation

The experiments were performed in the urinary bladder and rectal smooth muscles isolated from adult Wistar rats, weighing 250–300 g. The animals were anesthetized with Nembutal 50mg/kg i.p. and exsanguinated. The experiments were carried out in accordance with the national regulations and the Directive 2010/63/EU of the European parliament and of the Council (22 September, 2010) concerning the protection of animals used for scientific purposes.

Abdominal and pelvic cavity were opened and the urinary bladder and rectum were dissected out and immediately placed in cold Krebs solution (3 ºC), containing the following composition (in mmol): 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 surrounding tissue was dissected and longitudinal sections from both organs (approximately 8-10 mm long) were prepared.

The two ends of each preparation were tied with 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 placed in organ baths TSZ–04/01, containing Krebs solution, pH 7.4, continuously bubbled with Carbogen (95% O_2, 5%CO_2). The organ baths were mounted in parallel above an enclosed water bath, maintaining the solution temperature at 37 ºC. Preparations were placed under an initial tension (preload) of 1 g and allowed to equilibrate for at least 75 min (three periods: 15 min, 45 min and 15 min and two washes with Krebs solution between them). After the equilibration period, preparations were influenced by Ang II and AVP in a dose of 1 μmol (10^{-6} M), applied separately.

Recording of mechanical activity

Mechanical activity was digitized and recorded by using S.P.E.L. ISOSYS Advanced Software (Experimetria Ltd., Hungary). The conversion of the data for later analysis was performed with KORELIA-Processing and the analysis and graphic processing—with KORELIA-Dynamics computer programs [17, 18].

Chemicals and drugs

Ang II (Sigma-Aldrich) and AVP (Sigma-Aldrich) were solubilized in bidistillated water. All reagents for the preparation of Krebs solution were purchased from Sigma-Aldrich.

Data analysis and statistical processing

The recorded force-vs.-time curves were analyzed including calculation of amplitudes, integral force of tonic contraction, presented as area under the curve (AUC), as well as determination of time-parameters. The different phases of the peptide - induced tonic contractions, were clarified and analyzed by application of a time -parameter analysis, similarly to that made in the study of the skeletal muscle contraction [19].

The following time-parameters were defined (Fig. 1.): 1st half-contraction time (T_{hc}), 2nd half-contraction time (T_c–T_{hc}), contraction time (T_c), half-relaxation time (T_{hr}), contraction plus half-relaxation time (T_{chr}).

The duration of the interval for analysis of tonic contraction was defined from the beginning of the contraction, until the amplitude fell to 50% (Fig.1).

For a better analysis and understanding of the different phases of the induced SMC, a normalization of time-intervals was performed. All of the normalized time-intervals were calculated as a relative part from T_{chr}.

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

RESULTS

Amplitude and AUC urinary bladder

After an equilibration period, the isolated strips from urinary bladder displayed spontaneous activity with an amplitude of 0.30 ± 0.06 g (n=19). Time-parameters of peptide-induced tonic contraction are presented on Fig. 1.

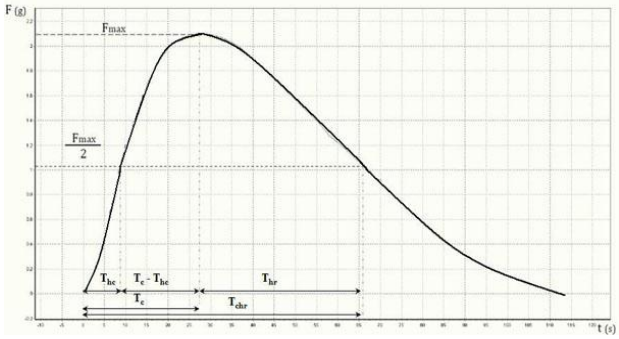

Fig. 1. Time-parameters of peptide-induced tonic contraction:**F_{max}** – maximal force of the smooth muscle contraction (SMC); **$F_{max}/2$** – half of maximal force of the SMC; **T_{hc}** – 1st half-contraction time: the time interval between the start of the SMC and $F_{max}/2$;**T_c - T_{hc}** – 2nd half-contraction time: the time interval between 1st half-contraction time and **F_{max}** ;**T_c** – time interval between the start of the SMC and F_{max} ; **T_{hr}** – time interval between F_{max} and $F_{max}/2$; **T_{chr}** – time between the beginning of the SMC until the amplitude fell to $F_{max}/2$.

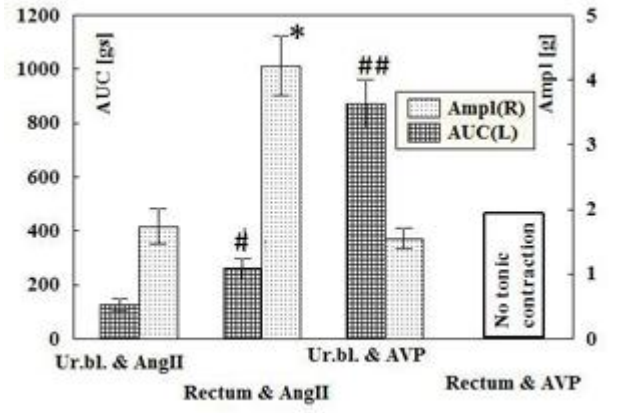

Fig. 2. Amplitudes and integral forces (presented as AUC) of Ang II- and AVP– induced tonic contractions from rat urinary bladder and rectum.

Ang II at a concentration 1 μmol induced tonic contraction with an amplitude of 1.74 ± 0.75 g (*n*=10) (Fig. 2, Fig. 3) and an integral force of muscle contraction of 126.6 ± 67.8 gs (Fig. 2).

AVP at the same concentration (1 μmol) induced tonic contractions with an amplitude of 1.55±0.5 g (*n*=18) (Fig.2, Fig. 3). The integral force of AVP-induced contraction - 871.5±287.8 gs was significantly greater than Ang II-provoked (Fig. 2).

Rectum

After a period of equilibration, the isolated longitudinal strips from rectum displayed spontaneous activity with an amplitude of 1.03 ± 0.5 g (*n*=8), which was significantly higher than the urinary bladder spontaneous activity. Ang II (1 μmol) led to significantly increased ($P < 0.05$) amplitude and integral muscle force of tonic contraction (4.22 **±** 1.12 g, 260 ± 92.7 gs) in comparison to bladder contraction (Fig. 2 and Fig.3). The application of AVP in a concentration of 1μmol did not cause onset of tonic contraction, but there was observed a noticeable increase in the frequency and the amplitude of the rectal phasic activity (Fig.3).

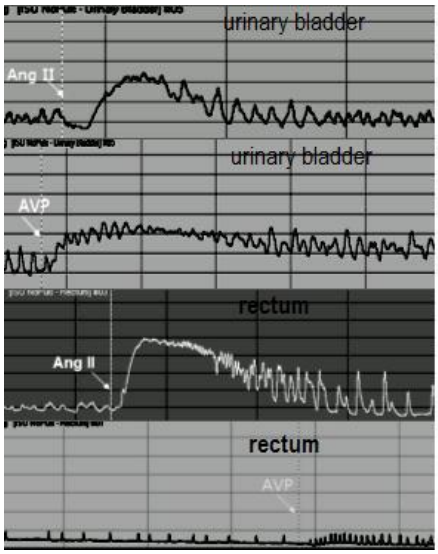

Fig. 3. Original recordings from S.P.E.L. ISOSYS Advanced Software (Experimetria Ltd., Hungary).

Time-parameter analysis

Regardless the reported differences in the amplitude and AUC of Ang II – induced rectal and urinary bladder contractions, such were not observed when time-parameter analysis was applied (Table 1). The initial time - parameter (T_{hc}) was significantly shorter in comparison to the subsequent interval (T_c–T_{hc}**)** and the parameter characterizing the process of relaxation (T_{hr}). In both of the Ang II – induced responses the interval for the second part of the development of contraction was bigger. Statistically significant differences between the absolute time-parameters of the Ang II- provoked contractions from the two preparations were observed only in the second half-contraction time (bladder - 19.5 ± 2.3 s and rectum - 29.5 ± 2.6 s). AVP-induced contraction was characterized by a significantly longer duration of all time-parameters compared to those induced by Ang II.

Table 1. Calculated time – parameters of Ang II – induced rectal and urinary bladder tonic contractions, as well as AVP – induced urinary bladder response. The application of 1μmol AVP did not led to appearance of rectal tonic contraction

Organ	Peptide	T_{hc} [s]	T_c–T_{hc} [s]	T_c [s]	T_{hr} [s]	T_{chr} [s]
Urinary bladder	Ang II	12.8±1.6	19.5±2.3	32.2±3.3	61.5±13.6	93.8±13.3
Rectum	Ang II	11.2±1.4	29.5±2.6	40.7±2.6	57.9±8.5	98.6±9.1
Urinary bladder	AVP	28.0±4.1	99.4±16.8	127.4±18.0	255.3±35.1	382.7±43.1

Normalized time - parameters

After the normalization of the time - parameters, the differences between AVP and Ang II - induced urinary bladder contractions disappeared. The normalized T_{hc} of AVP-induced bladder contraction was significantly less than that of Ang II-induced and represented 0.08 ± 0.01 and 0.16 ± 0.03, respectively (Fig. 4). The comparison of Ang II – provoked bladder and rectal responses revealed differences only in the second part of the contraction (0.23 ± 0.03 and 0.31 ± 0.03, respectively).

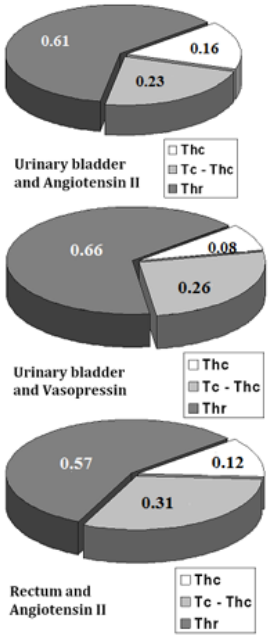

Fig. 4. Normalized time-parameters. All of the normalized time-intervals were calculated as a relative part from T_{chr}.

DISCUSSION

Ang II – and AVP- induced urinary bladder contractions: a detailed view and some assumptions

Ang II receptors have been discovered in detrusor of many species, including human and there is wide variation in the response to this peptide [1]. The application of Ang II and AVP in our experiments at a concentration of 1μmol led to development of tonic contractions, which confirms our previous investigations and available literature data for the effect of these peptides on urinary bladder contractile activity .[2, 12, 13, 20, 21].

The comparison of the results from the independent influence of Ang II and AVP on bladder activity demonstrates that the observed contractions were with approximately equal amplitude, but the developed integrated muscle force was significantly increased after the application of AVP. Time – parameter analysis indicates that Ang II causes contractions for a shorter period of time: the first and the second half – contraction time as well as half – relaxation time were significantly brief. AVP - induced response, which reached a noticeable maximal integrated force, is due to a prolonged achievement of the maxim and the subsequent much slower relaxation. The absence of significant differences in the normalized time - parameters, excluding the initial period of contraction, indicates that the bladder contractile responses to Ang II and AVP generally follow an identical pattern. This is precisely due to the activation of same signal transductional pathway by both peptides. Then what are the differences between the mechanisms of action of the two peptides that alter the muscle force and the absolute time – parameters? A possible explanation is that Ang II is metabolized and its derivatives are biologically active. Their effect, however, is opposite to that manifested by Ang II [22].

Also we can express the assumption that the probable reason for this difference is a consequence of the interaction of these two peptides with the ion channels of the plasmalemma. There is data for the impact of AVP over potassium channels of brain cells after fluid perscussion brain injury indicating that AVP blunted K_{ATP} and K_{Ca} channels [23]. It is known that smooth-muscle cells of the bladder have a number of potassium channels, including ATP-dependent K channels and Ca-dependent K channels [2]. Such data could be a possible explanation for the prolonged effect of AVP on SMC.

Ang II stimulates the activity of T-type voltage dependent calcium channels in vascular smooth

muscle cells [24]. We can assume that in the smooth muscle cells of the rat bladder is realized the similar effect.

Ang II – provoked rectal response. Comparioson with the urynary 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-} T_{hc} parameter, do not indicate significant differences. 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 it the rectum a local renin-angiotensin system has also been established [14]. It could be considered again that the locally generated metabolites of Ang II contribute for this pattern of the contraction process.

Does AVP have an importance for the motility of the rectum?

Dose-dependent effects of AVP on gastro-intestinal tract from different species were observed, but regarding the rectal musculature the information is unsufficinet and controversial [25]. AVP has been shown to increase the gastric and duodenal motility in humans and rabbits [15, 16], as well as colonic peristalsis [16], but the expression of the exact AVP receptors in intestine has not been examined yet [16]. Some authors have demostrated that AVP increase the gastro-intestinal motility via the oxytocin OT1 receptors, but the experiment is only for stomach and duodenum from rabbits [15].

In our study, the application of AVP does not significantly alter the characteristics of the spontaneous phasic contractile activity of the rectum. 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.

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ЕФЕКТИ НА АНГИОТЕНЗИН II И ВАЗОПРЕСИН ВЪРХУ СЪКРАТИТЕЛНАТА АКТИВНОСТ НА ИЗОЛИРАНИ ТЪКАННИ ИВИЦИ ОТ ПИКОЧЕН МЕХУР И РЕКТУМ НА ПЛЪХ

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(Резюме)

Целта на настоящото изследване е да се анализират и сравнят силата и времевите характеристики на Ангиотензин II (Анг II) и Аргинин-вазопресин (АВП) – предизвикани контракции на мускулни ивици от пикочен мехур и ректум на плъх, при проведени in vitro експерименти. Използвани са мъжки плъхове - линия Wistar, с тегло 250–300g. От пикочния мехур и ректума са приготвени лонгитудинални препарати, на които е въздействано самостоятелно с Анг II или АВП, в дози по 10^{-6} М. Записаните криви сила-време са анализирани посредством изчисляване на амплитуда на съкращението, площ под кривата (AUC), както и чрез определяне на някои времеви параметри. Анг II и АВП предизвикаха тонични контракции на пикочния мехур, със сходна амплитуда (съответно 1.74 ± 0.27 g и 1.55 ± 0.16 g), но с различни AUC. Отчетливи разлики се наблюдаваха при приложението на двата пептида върху гладко-мускулните препарати от ректум: Анг II предизвика тоничен отговор с амплитуда от 4.60 ± 0.42 g, докато АВП не промени съществено фазичната спонтанна активност на препаратите. Анализът по времеви параметри установи аналогично развитие на отговорите към Анг II и при двата вида органи. При пикочния мехур, наблюдаваните различия в Анг II – и АВП – медиираните контракции биха могли да се дължат на допълнително действие на Анг II – деривати и/или на взаимодействието на двата пептида с йонните каналчета на плазмалемата. Сходството във времевите параметри на Анг II – предизвиканите съкращения на пикочния мехур и ректума насочва към вероятен сходен механизъм на развитие на контракцията при двата органа. Липсата на тоничен отговор при ректума, след въздействие с АВП, навярно се дължи на различен тип рецептори или модификации на трансдукционната сигнална верига.