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AT2 RECEPTORS AND ANGIOTENSIN II-MEDIATED CONTRACTIONS OF GASTROINTESTINAL TRACT OF RATS

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ABSTRACT

Purpose: The present study was designed to compare the registered responses to Angiotensin II (Ang II) of different segments from gastro-intestinal tract (GIT) and to investigate the importance of AT2 receptor subtype in the development of smooth-muscle contraction by application of the specific AT2-blocker: PD123319. Methods: 10 male mature Wistar rats were used. Longitudinal smooth-muscle strips from GIT were prepared. The preparations were divided into 2 groups: the first was influenced by Ang II (1μmol) and the second - by PD123319 (100nmol) and 20 min after that - by Ang II (1μmol). Results: The presence of PD123319 led to increase of Ang II – provoked contractions: from 1.14 ± 0.13 g to 2.03 ± 0.37 g for the stomach and from 1.08 ± 0.16 g to 2.11 ± 0.16 g for the ileum. In the jejunum the effects of the blocker were opposite. The most significant differences were observed when blocking AT2 receptors in the rectum. Conclusion: The study indicates that AT2 receptors are of importance for the development of the GIT response to Ang II. They affect both phases of the smooth-muscle contraction.

Key words: Angiotensin II, AT2-blocker, Time-parameters, Smooth-muscle contraction, Angiotensin II receptors.

INTROUCTION

Angiotensin II is a multi-functional hormone that plays an important role in the regulation of blood pressure, cardiovascular homoeostasis, fluid and electrolyte balance (1, 2, 3, 4). The actions of Ang II are mediated by at least two receptor subtypes - AT1 and AT2 (5, 6, 7).

Most of the well-described actions of Ang II, such as vasoconstriction, stimulation of aldosterone release and promotion of cellular growth are all mediated by the AT1 receptor. (5). The signal transduction mechanism for AT1 receptors is well known. These receptors mediate Ang II effects by activating phospholipase A2, phospholipase C, phospholipase D and L-type Ca^{2+} channels and inhibit the adenylyl cyclase (5, 7, 8).

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Much less is known about the function of the AT2 receptor, but recent studies suggest that it may play a role in mediating anti-proliferation, cellular differentiation, apoptosis and vasodilatation (5). Although different signaling pathways have been assumed, for example activation of various phosphatases, cGMP - NO system (2, 5), their actual signal transduction is not quite elucidated.

In the alimentary tract, the role of rennin-angiotensin system has not been extensively investigated but recently the numbers of studies show the presence of the Ang II receptor subtypes at various locations along the GIT (1).

Even though the functional effects of Ang II in the GIT have been documented, it has not been established whether AT1 or AT2 receptors are responsible for the effects of Ang II peptides in the different areas of the alimentary canal.

Most of the effects of Ang II in GIT are associated with AT1 receptors, especially concerning the smooth muscle contractile activity (1, 9, 10, 11). Currently, the role of AT2 receptors in GIT is associated with the

exchange of water and salts, sodium hydrogen carbonate secretion in the duodenum (1) and the secretion of nitric oxide in pig's jejunum (2). 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 (4, 12), but as a factor for the smooth muscle relaxation they had been proved only for the internal anal sphincter (13, 14).

AIM

The present study was designed to compare the registered responses to Ang II of different segments from GIT and to investigate the importance of AT2 receptor subtype in the development of smooth muscle contraction by application of the specific AT2 - blocker : PD123319**.**

METHODS

Animals

10 male mature Wistar rats, weighting 250–300 g were used**.** The animals were anesthetized with Nembutal 50 mg/kg intraperitoneally and exsanguinated. Abdominal cavity was opened and the stomach, jejunum, ileum, colon and rectum 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, $NaHCO_3$—25.0, $MgSO_4$—1.2, $CaCl_2$—2.0, KH_2PO_4—1.2 and glucose 11.0.

The experiment was carried out 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.

Sample preparation

Longitudinal strips (approximately 8 mm long and approximately 2 mm wide and 0.5 mm thick for the stomach) 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(Experimetria, Ltd., Hungary), 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 °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.

Only the smooth muscle strips that developed spontaneous activity were used in this study.

Experimental protocol

After the equilibration, the preparations were divided into 2 groups: the first one was influenced only by Ang II (1 μmol) and the second one - by PD123319 (100 nmol) and 20 min after that - by Ang II (1 μmol).

Recording of the mechanical activity

Mechanical activity was digitized and recorded using ISOSYS-Advanced 1.0 software (Experimetria Ltd., Hungary). The conversion of the data for later analysis was performed with KORELIA-Processing program (15).

Chemicals and drugs

Ang II, PD123319, and the reagents for the preparation of Krebs solution were purchased from Sigma-Aldrich Chemie GmbH, Germany.

Data analysis and statistic processing

The duration of the interval for analysis of the tonic contraction was defined from the beginning of the contraction, until the amplitude fell to 50%. The recorded force-vs.-time curves permit determination of amplitudes and integrated force of contraction, represented by the area under the curve (AUC). The different phases of the provoked contractions from the various segments of GIT could be clarified and analyzed by application of a time-parameter analysis, similarly to that made in the study of the skeletal muscle contraction (16). Following time-parameters were introduced and examined: half - contraction time ($\mathbf{T_{hc}}$); contraction time ($\mathbf{T_c}$); half-relaxation time ($\mathbf{T_{hr}}$); contraction plus half-relaxation time ($\mathbf{T_{chr}}$). **(Figure 1)**

Analysis

Reported amplitudes, AUC and time - parameters of smooth muscle contractions were analyzed with KORELIA — Processing program (15). Obtained data were processed by the statistical program Statistica 8.0, StaSoft, Inc. and presented as mean ± standard error. A p-value ≤ 0.05 was considered to be statistically significant.

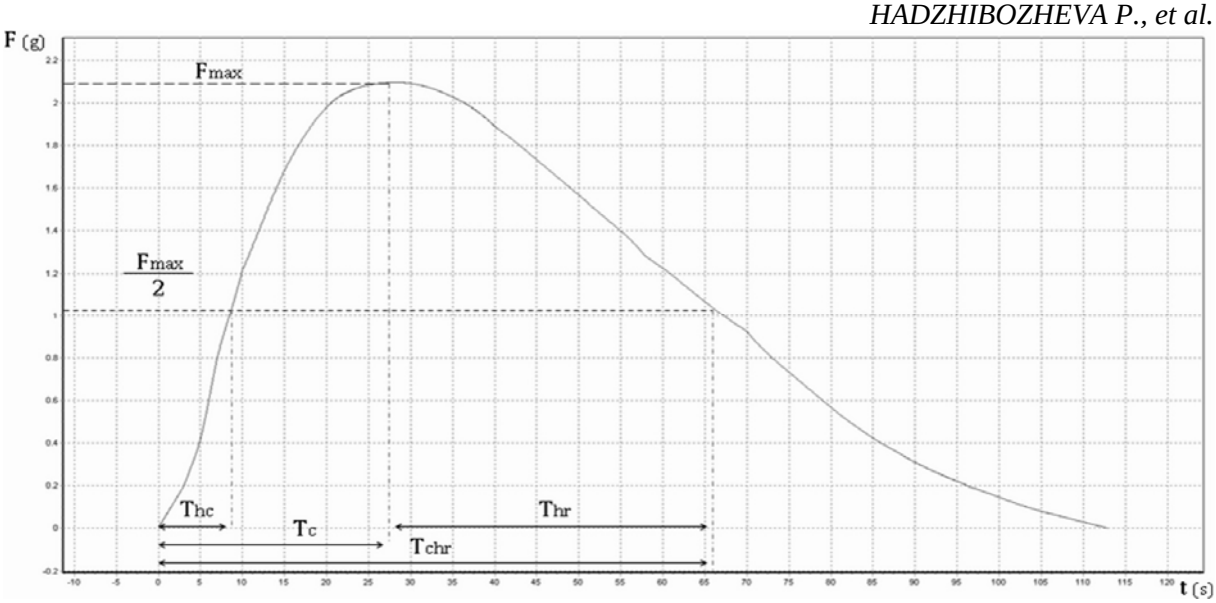

Figure 1. Smooth muscle contraction (SMC) - graph and time - parameters
Fmax – maximal force of the SMC
Fmax/2 – half of maximal force of the SMC
$\mathbf{T_{hc}}$ – half contraction time: time interval between the start of the SMC and Fmax/2
$\mathbf{T_c}$ – contraction time: time interval between the start of the SMC and Fmax
$\mathbf{T_{hr}}$ – half-relaxation time: time interval between Fmax and Fmax/2
$\mathbf{T_{chr}}$ – contraction plus half-relaxation time: time between the start of the SMC and Fmax/2

RESULTS

The estimated amplitudes and AUC of the smooth muscle contraction from the different preparations of GIT are presented on **Figure 2**. As can be seen, in the presence of AT2 blocker PD 123319, the Ang II - provoked stomach contraction is with significantly higher amplitude– 2.03 ± 0.37 g and AUC - 349, 23 ± 42.95 gs, compared to that, provoked by Ang II only (1.14 ± 0.13 g and 178.10 ± 19.62 gs, respectively). Similar changes are observed in the preparations of ileum - the group with PD 123 319 + Ang II displays statistically significant stronger contractions. The jejunal contraction however, is exactly the opposite - it appears that the amplitude and AUC of PD 123 319 + Ang II – mediated responses (0.47 ± 0.08 g and 24.04 ± 5.41 gs) are significantly lower than Ang II –mediated only (1.11 ± 0.14 g and 92.33 ± 8.02 gs). Preparations of rectum in the presence of AT2 blocker also demonstrated weaker responses to Ang II influence. Only the preparations from colon showed no statistically significant differences in the amplitude and AUC of contraction, despite the presence/ absence of PD 123 319.

The time-parameter analysis is shown on **Figure 3.** Ang II – mediated contraction of the ileum lasted significantly shorter (T_{chr} - 110.5 ± 4.79 s) when AT2 blocker was applied in comparison to that induced with Ang II only (T_{chr} - 141.08 ± 9.48 s). Conversely, the presence of PD 123 319 led to significantly shortening of T_{hc} and T_c parameters of the Ang II provoked contraction of the colon. The biggest differences were observed in the rectal responses to Ang II, where all of the time - parameters of the smooth-muscle contraction were significantly prolonged when AT2 blocker was applied.

DISCUSSION

A variation in the density of Ang II receptors in GIT has been mentioned from some authors (1, 12). There were changes in contractile activity when blockade of AT2 receptors was performed and in the various segments of GIT the observed effects varied. This suggests a different distribution and importance of AT2 receptor subtype along the alimentary canal. The response of the gastric and ileac preparations to Ang II in the presence of PD 123 319 was similar, which determines identical reactions of these two organs in regard to Ang II. According to Fandriks et al. (1), in these two organs only the presence of AT1 receptors was described, but it is interesting that the application of the AT2 receptor blocker led to an increased force of their responses to Ang II.

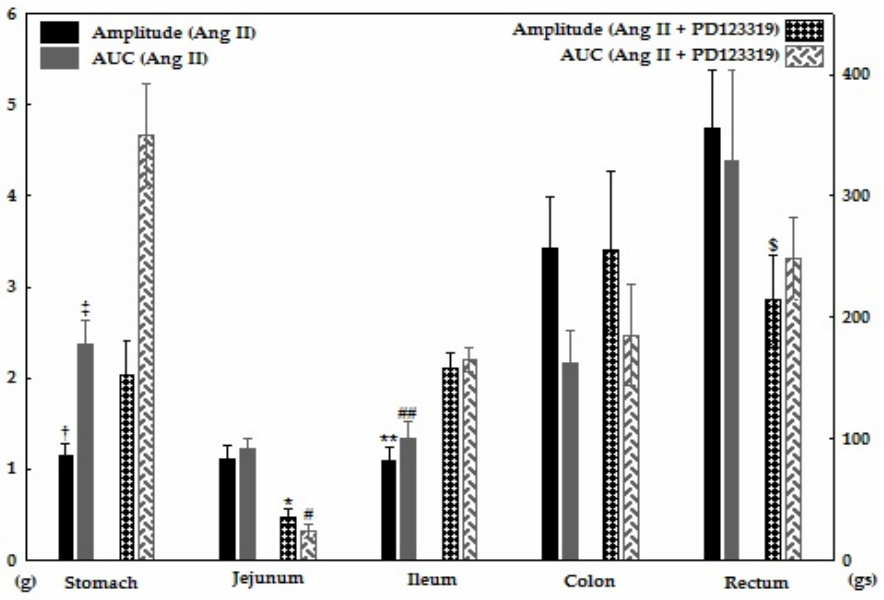

Figure 2. Integral forces (AUC) and amplitudes of smooth-muscle preparations from rat segments of GIT under the influence of Ang II (n=10) and Ang II added 20 min after PD123319 (n=10).
In the stomach and ileum, the application of PD123319 led to contraction with significantly higher AUC and amplitude. †P<0.05 vs. Ang II + PD123319 - mediated stomach contraction. ‡P<0.05 vs. Ang II + PD123319 - mediated stomach contraction. **P<0.05 vs. Ang II + PD123319 - mediated ileal contraction. ##P<0.05 vs. Ang II + PD123319 – mediated ileal contraction. In the jejunum there is also statistically significant difference among the two groups of preparations. *P<0.05 vs. Ang II-mediated jejunal contraction. #P<0.05 vs. Ang II-mediated jejunal contraction. The Ang II – induced rectal contraction in the presence of PD123319 was with significantly smaller amplitude. $P<0.05 vs. Ang II-mediated rectal contraction.

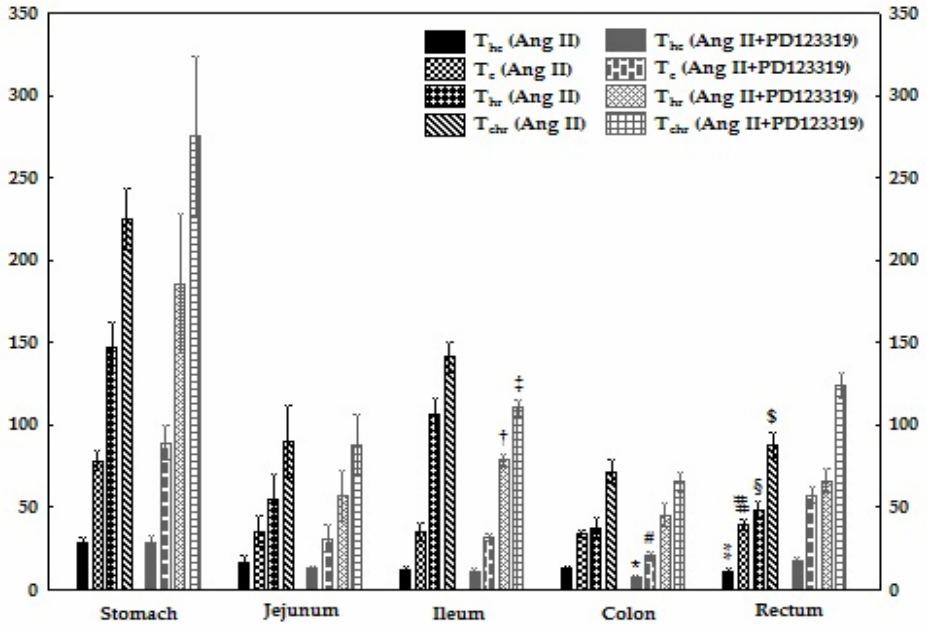

Figure 3. Time-parameters of smooth-muscle preparations from rat segments of GIT under the influence of Ang II (n=10) and Ang II added 20 min after PD123319 (n=10).
The presence of PD123319 decreased T_{hr} and T_{chr} of Ang II – provoked ileal contraction. †P<0.05 vs. Ang II-mediated ileal contraction. ‡P<0.05 vs. Ang II-mediated ileal contraction. T_{hc} and T_c of Ang II – induced colon contraction were statistical significant longer when PD123319 was added. *P<0.05 vs. Ang II-mediated colon contraction. #P<0.05 vs. Ang II-mediated colon contraction. All time - parameters of the rectal contraction were increased when PD123319 was applied. ** ## § $ P<0.05 vs. Ang II + PD123319 - mediated rectal contraction.

Although the importance of the AT2 receptors for the smooth muscle relaxation has been demonstrated only for the internal anal sphincter for the moment (13, 14), it could be assumed that these receptors in the stomach and ileum mediate a similar effect. In jejunal and rectal preparations, the presence of PD 123 319 led to an exactly opposite effect: a reduced response to Ang II. Probably the AT2 receptors in these organs may contribute to some extent to Ang II - mediated contraction.

Competing actions between AT1 and AT2 receptors have been discussed in the smooth musculature of rat small intestine (17) and this might be of importance for the power and the duration of Ang II – induced contractions. Perhaps not only the level of AT 1 and AT2 receptor expression, but also the interaction between the two receptor subtypes plays a role as a determinant for the magnitude of Ang II – mediated responses.

Time-parameter analysis of the contraction process revealed that AT2 receptors apparently could be important for different phases of the contraction. In the ileum, the blockade of AT2 receptors led to significantly shortening of the T_{hr} and T_{chr} parameters, thus further supporting the assumption for possible importance of this Ang II receptor subtype for the ileal relaxation. The fact that all the time - parameters of the rectal Ang II - stimulated contraction were statistically different from those of the contraction, induced by Ang II in the presence of PD 123 319, is interesting. It could be suggested that the AT2 receptors contribute to the rectal contraction and their blockade reduces not only the strength of the response to Ang II, but also significantly delays the time for the development of contraction and relaxation. Recently, Ang II is not considered to be the only biologically active product of the renin-angiotensin system, but physiological role has been also assigned to other Ang II fragments (18). This could also give some direction for a clarification of the obtained results. Furthermore, a local renin - angiotensin system or parts of it had been found in rat internal anal sphincter, rat stomach and rat small intestine (19, 18). It is possible the locally produced products in some of the organs from GIT, to modulate the Ang II - provoked contractions. Apparently not only Ang II, but also shorter peptides in the renin-angiotensin system, might contribute toward the changes in the smooth muscle activity of GIT segments.

CONCLUSION

The study indicates that AT2 receptors are of importance for the development of the GIT response to Ang II. This Ang II receptor subtype probably mediates different effects in the segments from the alimentary tract. The use of time - parameters significantly contributes to the analysis of the contraction process and permits a good comparison of the induced responses. It is obvious that the role of AT2 receptors on GIT tract needs further investigation.

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