Pharmacological analysis of imidazoline receptors in the gastrointestinal tract

PhD Thesis

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Budapest

2018

1. INTRODUCTION

During my PhD thesis I describe the effects of imidazoline receptor (IR) ligands on the gastrointestinal (GI) tract using various animal models (alcoholic ulcer model, chemically induced colitis model, inhibition of cholinergic activity of gastric fundus). The foundation of imidazoline hypothesis is that the central antihypertensive effect of α_2 adrenergic receptor (AR) agonist clonidine is not mediated by α_2 -ARs but by imidazoline receptors (IRs). Research has shown the use of IR ligands in many diseases, but some studies question the functional role of IRs. Furthermore, IR research is significantly complicated by the fact that IR ligands bind to α_2 -ARs with high affinity, so in case of effect, the role α_2 -ARs should always be excluded.

1.1. The IRs

Three major IRs were first isolated by radioligand binding assays and specific anti-IR antibodies. I1R is a pertussis toxin sensitive G proteincoupled receptor. which activation, phosphatidylcholine-sensitive phospholipase C cleaves phosphatidylcholine to phosphocholine and diacylglycerol. The latter is cleaved by the diglyceride lipase and then arachidonic acid is produced by monoglyceride lipase. Of this, prostaglandin E2 (PGE2) or other eicosanoids may also be formed, and diglycerides may also activate protein kinase C (PKC) β . The wide central nervous system (CNS) (medulla oblongata, rostral ventrolateral medulla, cortex, striatum, globus pallidum, hypothalamus) and peripheral occurrence (adrenal chromophile cells, platelets, glomus caroticum, proximal tubules of kidneys) of I1R have been described. In addition to its hypotensive effect, it is responsible for numerous effects based on its multiple distribution, it also reduces left ventricular hypertrophy, favourably affects the cellular building processes of the ventricular myocardium, and, among other things, enhances natriuresis, diuresis and reduces cholesterol levels as well.

I2Rs have also been identified in the periphery (pancreas, liver, prostate, platelets, colon epithelial cells) and CNS (cortex, striatum, glial cells). First, I2Rs were identified as allosteric binding sites of monoamine oxidase (MAO) (MAO-A and MAO-B), later I2Rs were also described as binding sites for brain creatine kinase and semicarbazide-sensitive amino

oxidase. I2Rs mediate among others antidepressant, analgesic and orexigenic effects as well.

The I3Rs have so far been identified only in the β -cells of pancreas, and during its activation, the release of insulin is increased, partly by the mediation of ATP-dependent K channels, partly in an independent manner.

1.2. Endogenous imidazoline ligands

Agmatine, which is an intermediate product of endogenous polyamine biosynthesis, is the first identified endogenous ligand of IRs. In addition to binding to I1R and I2R, it activates, for example, α₂-ARs, NMDA receptors, neuronal nicotinic acetylcholine (ACh) receptors, 5-HT3 receptors, and inhibits nitric oxide synthase (NOS) function as well as it has a neurotransmitter /neuromodulatory role. Regarding its incidence of tissue, it is found in highest concentration in the stomach, but it is also reported in the CNS (e.g. cerebral cortex, primary somatosensory and auditory cortex, periaqueductal area). Numerous effects of agmatine have been documented, such as it enhances the analgesic effect of morphine, inhibits morphine tolerance and. among other things, it has anti-inflammatory. antihypertensive, bradycardizing, antidepressant and anxiolytic effect.

 β -carbolines are formed by the condensation of indolamines and short chain carboxylic acids/aldehydes, and their principal representatives are harman, harmalan and norharman. The compounds are highly lipophilic; therefore, they are found mainly in the KIR. They have wide receptor binding, harman binds with significant affinity to I1R/I2R (slightly to I3R) benzodiazepine binding site, μ - and δ -opioid, 5-HT and muscarinic ACh receptors as well, and it activates NOS and inhibits MAO enzyme activity (mainly MAO-A inhibitor). Among the effects mediated by it, hypotensive, hypothermic, anorexigenic, anxiolytic, antidepressant and anticonvulsant effects can be highlighted.

1.3. The role of imidazoline ligands in the GI system

IRs were first identified by radioligand binding assays in the gastrointestinal tract of guinea pigs, rabbits, rats and humans. Subsequently, nischarin, an intracellular protein that binds to $\alpha 5\beta 1$ integrin and regulates

cell migration, but also presumably has a close functional relationship with I1Rs, has also been demonstrated in the rat GI system, thereby providing multiply proof about the presence of IRs in GI tract.

Controversial literature data are available about the relationship between IRs and gastroprotection. Some data indicate that agmatine mediates ulcerative effects while blended α_2 -AR/I1R agonists have proven to be moxonidine gastroprotective. The gastroprotective effect of agmatine was also documented in another publication, where the i.p. administered agmatin significantly reduced the gastrointestinal lesions on the ischemiareperfusion model via the PI3K/Akt signaling pathway. As the literature outcomes are controversial, we aimed to clarify how IRs affect gastric defence. By enhancing gastroprotection, it would be possible to further treat peptic ulcers (besides acid secretion reduction), after all, the inhibition of gastric secretion does not create adequate healing in some cases (e.g stress ulcer). In the central component of gastric mucus integrity, the role of dorsal vagal complex (DVC), hypothalamus, and NO, PGE2, PGI2 and CGRP as peripheral factors are important.

In the second major issue of my research, I examined the role of synthetic and endogenous IR ligands in the pathomechanism of DSS colitis, which is undiscovered in literature. Lot of researches examine the role of the IRs in the processes of inflammation. For example, that mixed α_2 -AR/IIR agonist clonidine and rilmenidine have caused an anti-inflammatory effects on epithelial oedema of rat paw, but the effect is linked to α_2 -AR. It has also been suggested that I1R is the homo- or heterodimer of the S1P receptor, and S1P1-3 receptors play an essential role in regulating the immune system. An example of this is that S1P receptor modulators reduce the severity of inflammation in different models like in colitis. Accordingly, we supposed that I1Rs of the S1P receptor family have anti-inflammatory effects and it can therefore be useful in the treatment of inflammatory bowel diseases.

The disorders of gut-brain interaction (DGBI) are the chronic diseases of the GI system, and one of their subtype is the functional dyspepsia (FD). The disease is postprandial associated with the hypomotility of the gastric antrum, decreased function of the migratory

motor complex, and in rare cases with the increased contraction of the gastric fundus. Therapeutic options for the treatment of the disease may be the relaxation agents of the gastric fundus, which can be achieved by the activation of NO-containing nitriterg fibres and by the inhibition of excitatory motor neurons (especially cholinergic neurons). Presynaptic IRs can inhibit the release of neurotransmitters, thus also the function of excitatory motor neurons. It demonstrates that presynaptic IRs inhibit the release of NA in researches of human atrial. It is suggested, that I1Rs and I2Rs are able to inhibit the cholinergic contractions. In addition, it is known from previous studies that clonidine can produce improvements in gastric accommodative disturbances. Given that the compound binds with significant affinity to IRs, the role of IRs in the mediation of the effect arises. There is a publication, which mentioned postsynaptic I2Rs, which have relaxant effect. Although this result contradicts most of the publications, even we still investigated the potential relaxant effect of I2Rs.

2. AIMS

- Investigating of the role of I1Rs and I2Rs in central gastroprotection. Identification of effect mediators and peripheral factors in the central gastroprotective effect of agmatine.
- Analysis of the role of I1Rs and α_2 -ARs in the DSS-colitis model.
- Investigating the function of IRs in controlling fundic contractility.

3. METHODS

The experiments were conducted under the ethical directives set up by the Ethics Committee of Semmelweis University, based on the Helsinki Declaration (EC Directive 86/609/EEC). The experiments were approved by the National Food Chain Safety and Animal Health Directorate of the National Government Office (license number: [PEI/001/1493-4/2015]; [22.1/606/001/2010]).

3.1. Applied compounds

During my PhD thesis, I used selective α_2 -AR, mixed α_2 -AR/IR and selective IR ligands as well. The used compounds with the subtype specificity and the distributor are indicated:

Selective α_2 -AR antagonists: yohimbin [α_2 -AR], BRL 44408 [α_{2A} -AR] and ARC 239 [$\alpha_{2B/C}$ -AR] (Tocris Bioscience)

Mixed α_2 -*AR/IR ligands:* clonidine (Sigma-Aldrich), moxonidine and rilmenidine (Tocris Bioscience) [α_2 -AR/I1R agonists], efaroxan [α_2 -AR/I1R antagonist] and idazoxan [α -AR/I2R antagonist (Sigma-Aldrich), agmatine [I1R, I2R, α_2 -AR ligand] and harman [I1R, I2R, I3R, α_2 -AR ligand] (Sigma-Aldrich/Tocris Bioscience).

Clonidine, moxonidine and rilmenidine are either mixed α_2 -AR/I1R ligands, but the clonidine has higher affinity to the α_2 -ARs than I1Rs. Moxonidine and rilmenidine has higher affinity to I1Rs than α_2 -ARs.

Selective IR ligands: AGN 192403 [I1R], 2-BFI [I2R], BU-224 [I2R antagonist] (Tocris Bioscience).

The compounds were solubilized in physiological saline, except for the water insoluble harman, which was dissolved in 1.7% acetic acid solution/ethanol.

3.2. Dosage of compounds

The compounds were administered intracerebroventricularly (i.c.v., 10 μ /animal), intraperitoneally (i.p., rat: 0.5 ml/100g, mouse: 0.1ml/10g) and orally (p.os; mouse: 0.1 ml/10g).

3.3. Alcoholic ulcer model

The acid-alcohol-induced ulcer model is an acid-independent ulcer model that can examine the function of factors that maintain gastroprotection and gastric mucosa integrity. The experiments were performed on 140-170 g Wistar rats, and the mucosa lesions were induced by intragastric administration of acidic alcohol (98 ml of absolute ethanol + 2 ml of concentrated hydrochloric acid) after 24 hours fasting. After 60 minutes of alcohol administration, animals were euthanized by CO_2 , their stomachs were cut along the large curve, and the lesions were evaluated by a 0 to 4-point system. The sum of the points of the ulcers gave the index of the given stomach lesion. The gastroprotective effect of the agonists was expressed by percent inhibition using the following formula: 100 - [ulcer index of the treated group/ulcer index of the control group] x 100. The control group received the vehicle of the given compound. The compounds used in the experiments were dosed i.c.v. 10 minutes and i.p. 20 minutes before the acidic alcohol administration, the antagonists were administered at the same time as the agonists. In the case of bilateral cervical vagotomy, the experiment, gastric mucosa samples were taken to determine the level of mucosal CGRP and somatostatin.

3.4. DSS-induced colitis model

One of the most frequently published models of chemically induced colitis models is the DSS-induced colitis model, in which the damaging agent is a sulphated polysaccharide polymer, the dextran sulphate sodium. The compound produces direct hyperosmotic damage to epithelial cells and it is associated with histologic (neutrophilic granulocyte infiltration, appearance of aberrant cryptos, enterocyte loss) and cytokine profile (mainly Th2 activation; IL-4, IL-5, IL-6, IL-13, TNF- α , TGF- β).

DSS-colitis was excreted by watering DSS solution, using 8-week wild-type (WT) and subtype α_2 -AR gene knockout (KO) C57Bl/6 mice in my experiments. During my PhD thesis, I introduced two different DSS colitis protocols, during one of which 2.5% DSS solution was given to mice for 7 days (medium density DSS-colitis model, for the analysis of possible protective effect of IRs), and in the remaining days the animals drank tap water. In the following protocol, I wanted to create a mild colitis (with the aggravating effect of the later α_2 -AR possible colitis), where 2% DSS solution was given to mice for 7 days. The body weight, nutrition and water consumption of the animals were monitored every day during the experiment. The colitis of animals was characterized by the DAI (disease activity index) (total score of 8 [2.5% DSS model] and 5 [2% DSS model], which was composed of the general state of the animals [2 or 1 points], the

consistency of their stool [3 or 2 points] and blood content [3 or 2 points]). The compounds were administered once or twice daily by p.os or i.p. The control group received tap water or a vehicle of the compound. 7 to 9 days after the start of experiment, animals were euthanized with CO_2 , and their colons were removed. The shortening of the colon length was expressed as a percentage compared to the control group. Total intestinal segments were removed from the distal colon to analyse myeloperoxidase (MPO) levels and cytokine profiles. In addition, serum IL-6 levels were also determined in some experiments.

In an additional experiment, I examined the locomotor activity of mice to quantify the sedative effect of clonidine. The experiments were recorded using the CONDUCTA behaviour and activity test, recording and analysis system, which is suitable for monitoring the motility of rodents. The locomotor activity was measured on 3 consecutive days (day 5, 6 and 7 of DSS treatment) at 3 different times (30 minutes, 3 hours and 6 hours after the injection of clonidine).

3.5. Electrical field stimulation induced gastric motility measurement

The experiments were performed on WT and different subtypes α_2 -AR KO C57BL/6 mice (20-25 g from both sexes). The gastric fundus was removed, and the prepared fundus strip was mounted on a 37 °C 5 mL, carbonated enriched Krebs solution containing organ bath (composition (mM): 118.0 NaCl, 25.0 NaHCO3, 4.7 KCl, 1.2 KH2 PO4, 11.0 glucose, 2.5 CaCl2, 1.2 MgSO4) between two electrodes, followed by 0.5 g pretension. Cholinergic contractions were induced by electrical field stimulation (EFS) with the following parameters: 1 ms pulse width, 200 ms pulse distance, 5 Hz supramaximal voltage per minute repeat. Contractions were recorded using the ISOSYS 8-channel in vitro assay. After 25-35-minute intervals to the organ bath and then, after recording the agonist dose-response curve, I added the test antagonist to the organ bath at a 10 μ M dose. This was followed by a 20-minute equilibration time.

3.6. In vitro methods

Measurement of tissue CGRP and somatostatin concentration was performed by radioimmunoassay (RIA) technique. Synthetic peptides were used for control, with a measurement limit of 2 fmol/ml for somatostatin and 0.2 fmol/ml for CGRP. The concentration of the two peptides was compared to the mass of the tissue, and its value was expressed in fmol/mg.

Qualitative (experiments with imidazoline ligands) and quantitative (clonidine experiments) analyses were made of the sections of distal colon samples containing the whole segmental intestinal segment. In the quantitative analysis, I took into consideration the extent of inflammation (max. 3 points), its depth (max. 3 points), the degree of crypt damage (max. 4 points) and the involvement of the mucosal area (max. 4 points). The total histological score (0 to 14 points) is the sum of the points of each parameter.

To determine the tissue MPO level of the distal colon containing the whole intestinal segment, the samples were homogenized in lysis buffer and their protein content was measured using a BCA method and I determined their MPO content by ELISA specific for murine protein. MPO levels were expressed as ng/mg of tissue protein (MPO content correlates with neutrophil granulocyte infiltration).

To determine the level of serum IL-6, the blood of animals was centrifuged, and the serum protein content was determined by the BCA method. IL-6 levels of the samples were determined by ELISA kit specific for murine protein.

The tissue cytokine levels of the whole intestinal segment from the distal colon were determined by mouse cytokine panel. Homogenization, centrifugation and protein determination of samples with BCA was followed by incubation of 600 μ g protein on a nitrocellulose membrane containing various anti-cytokine antibodies. Membrane-bound cytokines were detected by chemo luminescence, and the intensity of the light emitted by the samples at the given points was measured by a densitometric method which was normalized to the pixel density (10,000) of the reference points.

3.7. Statistics

Statistically significant difference was p<0.05. For statistical analysis, Student's t-test, one-way ANOVA method (Newman-Keuls and Holm-Sidak post hoc test), Mann-Whitney, Kruskal-Wallis test (Holm-Sidak post hoc test), Friedman test and two-way repeated measurement ANOVA method (Newman-Keuls post hoc test) were performed. The survival rate was analysed by Kaplan-Meier test. In case of gastric motility tests, the 50% effective concentration of the agonists (EC50) and their maximum inhibitory effect (Emax) were determined from the sigmoid concentration-response curves fitted with the Hill 4 equation.

4. **RESULTS**

4.1. Examination of the gastroprotective effect of imidazoline ligands

The endogenous IR ligand agmatine injected both i.c.v. (0.044 to 220 nmol/rat; bell-shaped effect) and i.p (0.01-50 mg/kg) inhibited the formation of alcoholic ulcers, but peripherally administered, it mediated a weaker gastric protection. During central delivery, a bell-shaped effect was seen, the background of which is the activation of receptors against gastric acid at higher doses (e.g. NMDA receptor). Further explanation is that agmatine increases the production of gastric acid at high doses, thereby increasing the direct harmful effect of alcohol and reducing gastroprotection.

In experiments with agmatine, a stronger gastroprotective effect was observed during central administration, therefore, during further experiments, the compounds were dosed i.c.v.

Subsequently, I studied the inhibitory effect of various antagonists for the receptors analysis of the protective effect of agmatine. In my experiments, the gastroprotective effect of i.c.v. injected agmatine was completely suspended by α_2 -AR/I1R antagonist efaroxan (4 nmol/rat), α_2 -AR/I2R antagonist idazoxan (160 nmol/rat) and the selective I1R ligand AGN 192403 (0.52 nmol/rat). However, selective α_2 -AR antagonist yohimbine only partially inhibited the protection.

To demonstrate the role of the IRs in the central gastroprotective effect, I examined the gastroprotective effect of selective IR ligands against acidic alcohol. Both the selective I1R ligand AGN 192403 (0.52 - 5.27 nmol used doses) and the selective I2R ligand 2-BFI (0.45 - 4.5 nmol) were dose-dependent gastric protective agents after i.c.v. administration.

Agmatine-induced mucous membrane protection was suspended by bilateral cervical vagotomy, while in experiments with the identification of peripheral factors, the pre-treatment of indomethacin (20 mg/kg per os) and NG-nitro-1-arginine (L-NNA) (3 mg/kg i.v.) both inhibited the gastroprotective effect of agmatine. In addition, tissue levels of CGRP and somatostatin decreased significantly due to the acidic alcohol treatment, but this effect did not occur in the agmatine-treated group.

4.2. The role of I1Rs and a2-ARs on the DSS-induced colitis model

To test the role of IRs, I first characterized the DSS-induced colitis model, using the 2.5% DSS solution in the first protocol for 7 days, and the experiments continued until day 9.

In my experiments, mixed α_2 -AR/I1R agonist moxonidine and rilmenidine (0.01-1 mg/kg i.p.) (whose has higher affinity to I1Rs than α_2 -AR), selective I1R ligand AGN 192403 (0.1-10 mg/kg i.p.), mixed α_2 -AR/I1R antagonist efaroxan (0.1 -10 mg/kg i.p.) neither exert any significant effects on inflammation parameters (DAI, body weight reduction, colon shortening) and did not affect the histological image of DSS colitis. As a result of DSS exposure, the MPO level correlating with tissue neutrophil granulocyte infiltration increased significantly, but none of IR ligands influenced this. IL-6 levels of blood serum were also increased by the DSS solution, but IL-6 level remained the same with saline, α_2 -AR/I1R agonist rilmenidine (0.1 mg/kg) and I1R ligand AGN 192403 (0.1 mg/kg) treated groups.

In experiments with endogenous IR ligands neither agmatine (10-100 mg/kg i.p.) nor harman (2.5-10 mg/kg per os) affected the course of DSS colitis. During the experiments, it was argued that the 1x daily dosage would not be enough to induce a substantive effect, so experiments were carried out with the addition of synthetic compounds twice a day. With twice a day doses of moxonidine, rilmenidine, AGN 192403 (0.1 mg/kg) and efaroxan (1 mg/kg), only moxonidine showed a significant increase in weight loss.

Based on the results of the IR ligands (data obtained with a daily 2x dosage moxonidine), it raised that the aggravation of DSS colitis may be mediated by α_2 -ARs, after all, IRs bind with α_2 -ARs with high affinity. Based on these, the aim was to create a less severe inflammatory protocol, therefore, in experiments with α_2 -AR ligands, mice used to drink 2% DSS for 7 days.

Some of the publication mention the role of the α_2 -ARs in the GI tract. The α_2 -ARs are localized on the enteral neuron, enterocytes like pre-or postsinaptycal receptors. The α_2 -ARs are important in the GI neurotransmission, in the electrolyte transport, in the motility and the central gastroprotection. The result are not obvious which are received in the models of animals, and humans regarding α_2 -ARs and inflammatory bowel diseases. The anti-inflammatory effect of α_2 -ARs is proven by that the α_2 -AR agonist dexmedetomidine inhibit the symptoms of TNBS-induced colitis. The mixed α_2 -AR/I1R agonist clonidine moderate the symptoms of ulcerative colitis (histological and endoscopic signs) when applied chronically. This observation is inconsistent to α_2 -ARs which are localized in macrophages and neutrophil granulocytes are responsible the release of proinflammatory cytokines. In consequence the α_2 -ARs

The daily administration of mixed α_2 -AR/I1R agonist clonidine (which has higher affinity to α_2 -ARs than I1Rs) (0.3-3 mg/kg i.p.) slightly aggravated the symptoms of DSS-induced colitis (DAI increased earlier, weight loss significantly increased). Exposure to DSS significantly increased the MPO content of the colon, but this was not influenced by clonidine as clonidine did not produce change in the histological score. It has been suggested that clonidine mediates the weight-loss effect via a direct/indirect (sedative) mechanism. Locomotor activity decreased in the highest clonidine doses treated animals (3 mg/kg i.p.), but it was relatively short period and after 6 hours the effect was eliminated. Consequently, the sedative effect (consequent reduced consumption of food) is probably not responsible for weight loss.

In the followings, I conducted my studies with different α_2 -AR subtype KO mice to identify α_2 -AR subtype transmitting the effect of clonidine. Based on my results, the aggressive effect of clonidine on α_{2A} -AR KO mice did not appearenced, while in α_{2B} - and α_{2C} -AR KO animals, clonidine had similar effects as in WT mice. My experiments were confirmed by selective α_{2A} -AR antagonist BRL 44408 (3 mg/kg i.p.) results, so inhibition of α_{2A} -AR significantly reduced the symptoms of colitis and reduced weight loss. This protective effect disappeared when combined with clonidine.

Finally, the anti-inflammatory effect of α_{2A} -AR antagonist BRL 44408 was also demonstrated at the level of cytokines and chemokines. BRL 44408 significantly reduced the CXCL13 level, which organizes with CXCR5 chemokine receptors the B cells in lymph nodes and lymphoid tissues and regulates the segregation of T and B lymphocytes. In addition, CCL3 decreased slightly which plays a role in acute inflammatory conditions in the accumulation and activation of polymorphonuclear leukocytes. In addition, BRL 44408 moderated the CXCL2 cytokine level, which is chemotactic to polymorphonuclear leukocytes and hematopoietic stem cells. The increase in GCS-F levels caused by the DSS solution decreased due to the α_{2A} -AR antagonist effect, which is likely to have an indirect cause, i.e. the BRL 44408 decreased the severity of the colitis, thereby reducing blood loss, thus moderating the rate of haematopoiesis. In addition, BRL 44408 reduced the tissue MPO level. Besides my results, the interesting thing is that the increase in the tissue inhibitor of metalloproteinase-1 (TIMP1) was induced by DSS solution and it further increased due to BRL 44408. Matrix metalloproteinases (MMP) are the main participants in the degradation of extracellular matrix components both in physiological and pathophysiological conditions (including colitis). One of the inhibitors of the MMPs function is TIMP1, so it is possible that TIMP-1 activity increased by BRL 44408 will shift the balance to TIMP, thereby improving the healing process and reducing tissue damage. Similar TIMP-1 mediated proteins have been described for Crohn's disease with infliximab treatment.

4.3. Investigating the role of IRs in controlling the fundus contractility

In the third major topic of my research, the roles of I1Rs and I2Rs were investigated in the case of the electrical field stimulated contraction in mice fundus. The cholinergic nerve endings were activated by the stimulation parameters used in the present experiments. My aim was to inhibit the excitatory motor neurons, thus causing the fundus relaxation and improving accommodation.

The α_2 -AR/I1R agonist clonidine, moxonidine and rilmenidine also (1 nM to 1 μ M/10 μ M) inhibited EFS-induced gastric contractions dosedependently in both WT, α_{2B} - and α_{2C} -AR KO mice. Of the tested agonists, the inhibitor effect of clonidine was highest which was followed by moxonidine and rilmenidine. Inhibition of cholinergic contractions in α_{2A} -AR KO mice was only found at the highest concentration used for clonidine (1 μ M) and moxonidine (10 μ M).

The inhibitory effect of the three mixed α_2 -AR/I1R agonist compounds on gastric contractions was reduced in the WT mouse by α_2 -AR/I2R antagonist idazoxan and selective α_{2A} -AR antagonist BRL 44408. The selective I1R ligand AGN 192403, the selective I2R antagonist BU-224 and the $\alpha_{2B/C}$ -AR antagonist ARC 239 did not influence the effects of clonidine, moxonidine and rilmenidine.

Further experiments with endogenous IR ligands were performed. Based on the results obtained, agmatine did not exert any significant effect on cholinergic gastric contractions. In contrast, the harman on WT or α_{2A} -AR KO mice produced a slight but significant contraction inhibition, but the two curves were different for the two mouse strains. The inhibitory effect of harman was neither suspended on the I1R ligand AGN 192403, I2R antagonist BU-224 nor on the α_2 -AR/I2R antagonist idazoxan. Subsequently I examined the effect of the selective I2R ligand 2-BFI, which resulted in a similar result (both in WT and α_{2A} -AR KO mice, it inhibited gastric contractions). At lower concentrations (10 nM - 1000 nM), the inhibitory effect was significantly different in the two strains, but at the highest dose (10 μ M) this difference disappeared. Among the investigated antagonists (AGN 192403, BU-224, idazoxan, BRL 44408, ARC 239), they did not suspend the effect of 2-BFI in WT mice.

5. CONCLUSIONS

- The endogenous IR ligand agmatine is gastroprotective also peripherally and centrally administrated. The central localization I1R and I2R also mediate gastric protection. For the central gastroprotective effect of agmatine, I1R and I2R are partially responsible, and efferent vagus fibres play an important role in the protection. Among peripheral factors, gastroprotection is mediated by NO release, PG synthesis, CGRP and somatostatin as well.
- I1Rs do not participate in regulating the course of DSS-colitis (taking into account the macroscopic, microscopic images, MPO and IL-6 levels as well). Activation of α_2 -ARs slightly aggravates the initial phase of DSS-induced inflammation, and their inhibition has significant anti-inflammatory activity. The activation of the α_{2A} subtype of α_2 -ARs is responsible for the proinflammatory effect.
- The contraction inhibitor effects of mixed α 2-AR/I1R agonists are mediated by α_{2A} -ARs in the fundus, and neither the I1Rs nor the I2Rs are involved in the effect. For endogenous IR ligand harman and for selective I2R ligand 2-BFI, mild contraction inhibition is presumably not mediated by IRs but by other receptors.

6. OWN PUBLICATIONS

Own publications in connection with the PhD dissertation:

Feher A, Toth VE, Al-Khrasani M, Balogh M, Lazar B, Helyes Z, Gyires K, Zadori ZS. Analysing the effect of I1 imidazoline receptor ligands on DSS-induced acute colitis in mice. Inflammopharmacology, 2017; 25: 107-118. [IF: 3.304]

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