Central Glutaminergic Influences on Viscero-visceral Inhibitory Reflex During Colonal Descending Distension in Ovine Model

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Abstract
The aim of presented study was to examine the participation of group I metabotropic glutamate receptors (mGluRs) in development and maintenance reticulo-ruminal motility inhibition caused by visceral pain caused by colonic distension (CD) of different degrees provoked in ovine. Motility inhibition was qualified as a vegetative symptom of the nocifensive effects (viscero-visceral inhibitory reflex). The experiment was carried out on 12 female sheep, Polish merino, in the anoestrus period. A five min CD episode provokes a statistically significant reticulo-ruminal motility inhibition. Intracerebroventriculary (i.c.v.) administration of the group I mGluRs antagonist DL-AP3 10 min prior to CD, prevents the visceral pain development. Practically all pain symptoms were easy to observe and quantify. These symptoms were recurrently appearing in animals suffering colon distension; however, the intensity was smaller than in the case of duodenal distension. It suggests that this model of colic is not only less invasive but also less painful for animals. In conclusion, the development and maintenance of reticulo-ruminal motor inhibition of colonic distension are dependent on group I mGluRs receptor activation in the CNS and that these receptors play a crucial role in modulating the acute colonic pain (experimental colic). This less invasive in vivo method may be useful for testing the efficacy of potentially analgesic xenobiotics in small ruminants.

1 Introduction
It is well known that experimental and clinical duodenal and colon distension (jejunal colic) provoked a number of different behavioural and clinical symptoms as well as neuroendocrinial changes. Visceral pain produced by intestinal colic is associated with the appearance of vomiting, stretching, dental rasping as well as other symptoms of vegetative pain. Tachycardia, hyperventilation, bleating, defecation, urination, hyper- or hypoalgesia, cortisolemia, catecholaminemia and reticulo-ruminal activity inhibition (viscero-visceral inhibitory reflex), final bloat (not easy to treat) and reduction of the animal welfare are also observed in animals suffering colic. In pain signal transduction and pain sensation metabotropic glutamate receptors (mGluRs) play a significant role.

mGluRs can be divided into two groups according to the mode of action: ionotropic (forming ion channel pore) and metabotropic (indirect activation of ion channels via signalling a cascade involving protein G). Glutamate receptors are located primarily in the central nervous system but also exist on the dendrits of postsynaptic cells, which are present e.g. in the intestine. mGluRs are also present in the sensation neurons projecting to CNS.

The aim of this study was to assess the effects of DL-AP3 in mechanical hyperalgesia caused by a colon distension in sheep.

2 Material and Methods

2.1 Preparation of animals
The experiment was performed on 12 mature crossbred ewes, Polish merino sheep of 40-45 kg B.W., being in the anoestrus period. Food was withdrawn 24 hours prior to the experiment.
Analgesia was initiated by i.m. ketamine (Calypsovet, 20 mg x kg\(^{-1}\) B.W., GEDEON RICHTER, Budapest, Hungary) administration followed by anaesthesia – after 15 min i.v. infusion of pentobarbital (Vetbutal, 20 mg kg\(^{-1}\) B.W., BIOWET, Pulawy, Poland). All animals, during unconsciousness, had a T-shaped silicone cannulas (inside diameter of 21 mm) inserted into the dorsal sac of the rumen, using the techniques previously described\(^2\). Simultaneously, under the same general anaesthesia/analgesia a permanent stainless steel cannula was inserted into the lateral ventricle of the brain, using the stereotaxic method described by Kania et al. (2006)\(^8\). Animals had free access to hay and water, except during the experimental period. The cannulas were cleaned after every experiment and occluded with a stainless steel stylette.

The experiment was carried out in 4 stages (groups, each of 6 animals) according to the technique previously described for L-AP3\(^{11}\).

2.2 Mechanography

The reticulo-ruminal contractions were analysed using the electric tensometric recorder PIT 212 (COMT, Białystok, Poland). The analysis of mechanograms and calculations of results were performed just after the electromyographic recording\(^{11}\). The frequency of reticulo-ruminal contractions was determined by the number on the mechanograms with 5 min intervals prior and after colonal distension accordingly to the earlier study\(^5\).

2.3 Drugs

In the present experiment, the following drugs were used: racemate DL-2-Amino-3-phosphonopropionic acid (DL-AP3), a nonselective group I metabotropic glutamate receptor (mGluR) blocker – (USP grade – A154, SIGMA-Aldrich), heparin (Heparinum – POLFA, Warsaw, Poland); reduced glutathione (Glutathione, Ethylester, SIGMA-RBI), procaine (2% solution, Polocainum hydrochloricum – POLFA, Warsaw, Poland). DL-AP3 racemate was dissolved in 0.9% NaCl. The lack of effect of the solvent was determined in preliminary experiments. Two days before the planned experiment, a silicone cannula was inserted into the external jugular vein under local anaesthesia - s.c. injection of 2 ml of procaine (Polocaine HCl, POLFA, Warsaw, Poland). In order to determine the concentration of cortisol, blood samples were collected in 10 ml test tubes containing heparin and after centrifugation the plasma was stored at -20°C until time of analysis. In animals subjected to colonal distension, blood samples were collected at the same time schedule and in the same manner (Fig. 1). In animals administered DL-AP3, blood collections were performed according to the same schedule as in the group of the control animals (100 μl of 0.9% NaCl).

2.4 Statistical analysis

Statistical analysis of the results was performed according to the earlier study\(^5\). The results are presented as x SEM. P value, less than 0.05 were considered statistically significant (P < 0.05) in all tests.

The experiment was performed in accordance to the specific national laws of animal protection (National Law for Animal Protection – 1997, Dz. U. 23 XI; Permission of 3rd Local Ethical Commission No 9/2001 issued 11.01.2001). After the experiment completion all animals were euthanized using Morbital (BIOWET, Drwalew, Poland).

3 Results

In the control group during 30 min of observation, no significant differences in the reticulo-ruminal motility were observed. Also intracerebroventricularly (icv) infusion of 100 μl 0.9% NaCl or DL-AP3 racemate in the doses 4.0, 8.0 and/or 12.0 mg in toto also did not significantly change the tested parameters. A mean frequency of rumen contractions was 5.9±0.4 c-5 min\(^{-1}\). After placing the balloon in a colon 40 cm from the anus constrictor 30 min from the planned CD, no significant changes in the number of reticulo-ruminal contractions were noticed. Mechanical distension of colon walls caused highly significant inhibition of the reticulo-ruminal motility.

3.1 The influence of icv infused saline upon reticulo-ruminal motility

Intraventricular infusion of 100 μl of 0.9%NaCl in the control animals (group I) during the first minute and 10 minutes before 0 time did not cause any significant changes in the reticulo-ruminal motility (changes in the range of 0.25 – 0.5 c.min\(^{-1}\)) in sheep during 120 min of observation. These results confirm the experimental data obtained earlier after a 5 min episode of duodenal wall distension\(^12\).

3.2 The impact of CD on reticulo-ruminal motility

The CD provoked reticulo-ruminal motility inhibition (from to 3.0±0.25 c-5 min\(^{-1}\) comparing to 5.0±1.0 in the control animals, during 60 min after the CD episode; p<0.01) (Fig 1). Mechanical distension of the colon causes an almost immediate drop of the number of reticulo-rumen contractions. Already during CD, a number of counts was reduced to about 40% of the that observed among the control animals. In 4 out of 6 animals a total lack of motility lasting 4 to 6 min was observed. A statistically significant inhibition of contractions frequency (an average 59.3%) was still observed during a period of 100 min following the CD termination (p<0.05). The amplitude of contractions during CD was also reduced by 66% as compared with the untreated animals, during 30 min after the CD termination (unpublished data).

3.3 The influence of DL-AP3 premedication on reticulo-rumen motility in animals with/without CD

A one min icv infusion of the racemate DL-AP3 in the dose of 4.0, 8.0 or 12 mg in toto, did not have any significant impact on the reticulo-ruminal contraction number (Fig 2). The inhibitory effect of DL-AP3 intracerebroventricularly was significantly reduced to 40% of that observed among the control animals.
CD on the frequency of reticulo-ruminal motility was significantly suppressed in the group of animals pretreated with DL-AP3 (3.0 and 5.25 c-5min⁻¹, respectively) (Fig. 3).

The lack of experimental data concerning the analgesic effect of mGluR receptor antagonists from group I in the visceral pain in sheep caused great difficulties in the explanation of results obtained in ruminants. However, it is known that spinal mGluRs from group I and II are of a great specific importance in the transduction of nociceptive stimulations in postinflammatory hyperalgesia conditions via the spinal transmission.

Fig 1: Comparative analysis of different colonal distension degree influence (CD150 and CD200) on reticulo-ruminal contractions frequency/5 min in comparison to control value (x±SD, n=6, a, b – different letters indicate statistically significant differences at p=0.05)

Fig 2: Comparative analysis of different DL-AP3 doses (4, 8, 12 mg/animal) influence on reticulo-ruminal contractions frequency/5 min in comparison to control value and colonal distension (CD 200)

4 Discussion

One minute intraventricular infusion of DL-AP3 in sheep prevented the appearance of the vegetative symptoms of pain (reticulo-ruminal motility inhibition, tachycardia, hyperventilation), neuroendocrine symptoms (increase of plasma cortisol and catecholamines concentration)

Physiopharmacology of visceral pain was elaborated on a model of mechanical nocifensive impulse emitted by duodenum and/or colon distension in rodents. Our results indicate that stretching of the intestine wall is correlated with the contraction amplitude of both duodenum and colon. It is also an important impulse for the intestine motility. In the contractility response of the mouse intestine wall to mechanical stimulus nerve regulation is implicated.

A distension of the colon wall, similarly to the earlier described duodenum distension, every time caused a significant retardation of viscerovenous inhibitory reflex, which was probably caused by the sympathetic-adrenal system stimulation and adrenal catecholamines release to the circulatory system and also a significant increase of plasma cortisol concentration (stimulation of hypothalamic-pituitary-cortico-adrenal axis). The action of mechanical pain stimulus in the intestine was qualified as a strong general stressor which released classical defensive reactions of the organism. Such reaction was prevented by the previous (10 min before CD) icv premedication with DL-AP3 racemate administered in 3 different doses. This premedication also prevented the appearance of such symptoms as tachycardia, hyperventilation as well as behavioural symptoms of intestinal pain. It proves that DL-AP3 only reveal its peripheral actions but also central ones, mainly by the inhibition of metabotropic glutamate receptors activity in motivational structures and nociceptive afferent pathways transmitting mechanical impulses, such as distension of intestines walls, from the periphery to a higher structure of nervous system.
In mammals, there are two distinct spinal afferent nerve tracts that can potentially transmit sensory information from rectum and distal colon (sigmoid) to the spinal cord. They are known as lumbar colonic nerves (LCN) / lumbar visceral ones and sacral colo-rectal pelvic nerves. It was not stated until now, which of these pathways is more important in the detection and transmission of visceral pain from colon and/or rectum. The latest results of Feng et al. revealed that in mice these two separate sensory nerve tracts are distinguished by the presence of at least 5 different classes of afferent fibres each of which reacts selectively and independently. In principle, it is not claryfied yet, whether colo-rectal pelvic pathway is the first of all nerve tracts with a low excitability treshold, which reacts to mechanical stimulation of small intensity. The lumbar splanchnic pathway is probably the main path that reacts at impulses of a high threshold carrying only a small amount (10%) of stretch afferent fibres sensitive to distension.

The presence of metabotropic glutamate receptors in dorsal horns of the spinal cord neurons which evokes that intraventricular/intrathecal application of the specific antagonists of mGluR1 reveals an analgesic activity both in rodents and large animals. In rats a knockdown of the spinal mGluR1 alleviates pain and restores opioid efficacy after the nerve injury. Recently published results prove that mice with deletions of endothelin-3 (ET-3 gene) in rectum, the model of Hirschsprung’s disease, loss selectively the ability to feel visceral pain. Qi et al. suggest a special participation of voltage gated Na+ and Ca2+ channels in feeling visceral pain whereas the application of these canal antagonists can contribute to new possibilities in visceral pain therapy.

Results of the presented study indicate that DL-AP3, non-specific mGluR1 receptor antagonist, prevented viscerovisceral inhibitory reflex inhibiting transmission of nociceptive impulses evoked by 5 min mechanical CD in sheep. DL-AP3 can be recommended in alleviating the intestinal colic symptoms in sheep assuming that similar effects of this drug will be observed in the case of its peripheral use.

5 Conclusions

It was demonstrated that icv premedication by DL-AP3 prevented viscerovisceral inhibitory reflex and pain provoked by mechanical colonal distension in sheep. Perhaps, this drug can be recommended in diminishing the intestinal colic symptoms after peripheral administration.

6 Conflict of interests

None

7 Author’s contributions

All authors carried out literature review and draft the manuscript. All authors participated in collection of data and arranged in tabular form. All authors read and approved the final manuscript.

8 References


