Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2014, Volume 2, Issue 12
ISSN: 2321–3647(online)

Establishment of Model of Visceral Pain due to Descending Colonal Distenstion and its Behavioural and Neuroendocrinial Assessmment before and after $mGluR_1$ Antagonist (L-AP3) in sheep

Bogdan F. Kania[1]*, Danuta Wrońska[2], Mikołaj A. Gralak[3]
1. University Veterinary Medicine JU-AU Center, Faculty of Animal Sciences and Biology, Department of Biotechnology, Hugon Kollataj University of Agriculture in, Cracow, Poland
2. Department of Animal Physiology and Endocrinology, Faculty of Animal Sciences and Biology, Hugon Kollataj University of Agriculture in Krakow, Cracow, Poland
3. Department of Physiological Sciences, Faculty of Veterinary Medicine, Warsaw University of Life Sciences - SGGW, Warsaw, Poland.

ABSTRACT

The present study examined contribution of mGluRs to the development and maintenance of changes in behavioural and clinical symptoms, blood concentrations of cortisol and catecholamines during visceral pain produced by descsending colonal distension (CD). Experiments were carried out in fourth stages (each of six sheep). Every experiment was performed simultaneously on two unfed animals, which were placed in two individual metabolic cages at one week intervals. Blood was collected 30 min prior to the experiment, at 0 time, and 5, 10, 15, 30, 60 and 120 min after intracerebroventricular (*icv*) administration of the $mGluR_1$ antagonist: L-2-Amino-3-phosphonopropionic acid (L-AP3, 0.2, 0.4 and/or 0.8 mg *in toto*), 10 min before, blocked the development of visceral pain symptoms and neuroendocrine changes in the plasma of sheep. This data demonstrated that the development and maintenance of the visceral pain symptoms of CD is dependent on activation of $mGluR_1$ in central nervous system (CNS) and that these receptors play a crucial role in modulating acute jejunal (colic) experimental pain It can be concluded that the $mGluR_1$ antagonists prevent behavioural, clinical and neuroendocrine symptoms of visceral pain. They can be possibly used in cases of acute visceral pain, especially, in combination with opioid agonists. Their simultaneous administration allows to minimise dose of opioids and slows down dynamics of the development of tolerance. This knowledge can be also useful in paliative medicine.

Keywords: L-AP3 (L-2-amino-3-phosphonopropionic acid), Descending Colonal Distension, Catecholamines, Cortisol, Sheep

*Corresponding Author Email b.kania@ur.krakow.pl
Received 16September 2014, Accepted 28September 2014

Please cite this article as: Kania BF *et al*., Establishment of Model of Visceral Pain due to Descending Colonal Distenstion and its Behavioural and Neuroendocrinial Assessmment before and after $mGluR_1$ Antagonist (L-AP3) in sheep. American Journal of Pharmacy & Health Research 2014.

INTRODUCTION

Glutamatergic receptors have complicated molecular structures comprehensive to excitatory amino acids (glutamate, aspartate, glycine). They include two families: ionotropic (NMDA) divided into three classes (NMDA, AMPA and kainate) and metabotropic glutamate receptors (mGluRs). Eight subtypes of mGluRs has been already identified. They have been classified into three groups on the basis of the amino acid sequence similarity and of transduction mechanisms.[1] Group I of the mGluRs includes subclasses $mGluR_{1A,\ 1B,\ 1C}$ and $mGluR_{5A,\ 5B}$, which activate phospholipases and induce synthesis of IP_3 as a second messenger and DAG. Group II of the mGluRs consists of $mGluR_2$ and $mGluR_3$. To group III of the belong following subclasses: $mGluR_{4A,\ 4B}$, $mGluR_6$, $mGluR_{7A,\ 7B}$ and $mGluR_8$. All receptors of groups II and III inhibit activity of cAMP-ase in recombined expression systems and in brain they modulate binding of protein G, uncaging PIP2-dependent K^+ channels (GIRKs) or voltage gated Ca^{2+} channels (VGCC)[2]. Majority of the mGluRs belonging to the group I, increase excitation of cells by inhibition of the activity of potassium channels. Their inhibitory action resulting from the activation of small conductance (SK)-type Ca-activated potassium channels was also described[3]. Expression of mGluRs of groups I and II was found in structures of CNS closely related with nociception: spinal cord superficial fields, responsible for pain filling or modulation of neurotransmission in the peripheral receptors (all main groups of mGluRs). The highest expression of group I receptors ($mGluR_{1,\ 2,\ 3}$ and $mGluR_5$) was revealed in neuronal plexuses of dorsal horns of spinal cord, as well as in peripheral endings of these neurons (presynaptically).[4,5,6,7] Receptor proteins of $mGluR_{4\ and\ 7}$ were found in the presynaptic endings of sensory neurons of dorsal horns of spinal cord.[8] In afferent endings of nociceptive nerves of dorsal horns, an immunoreactivity against $mGluR_7$ was estimated[9]. In different thalamus nuclei (with neural projection of spinal-thalamic tract from dorsal horns of spinal cord) the different mGluRs were also stained: $mGluR_1$ - [10], $mGluR_{2\ and\ 3}$ – [11], $mGluR_4$ - [12], $mGluR_5$ - [13], and $mGluR_8$ – [14]. Particularly strong expression of these receptors in that region was found for $mGluR_{1\ and\ 3}$[15]. The important places for the projection of nociceptive receptors of dorsal horn of spinal cords is reticular formation and periaqueductal gray (PAG), which is one of the most important centers for efferent analgesic signals the spinal cord. The expression of $mGluR_5$ in the PAG was confirmed.[16] Frontal lateralo-ventral region of medulla (RVM) is important for efferent analgesic processes. Also in other structures involved in transmission of pain information e.g. amygdala and association cortex, there are mGluRs, which are currently intensively studied. This short literature review shows the

possibility of significant role of the mGluRs in both peripheral as well as in central transmission of nociceptive stimuli and generally in pain phenomenon/felling. It is known that peripheral receptors of group I ($mGluR_{1 \text{ and } 5}$) participate in the occurence of non-evoked pain reminiscence and secondary hyperalgesia provoked mechanically by intraarticular or intradermal injection of carrageenin causing post-inflammatory pain.[17] Peripheral application of the mGluR antagonists revealed antinociceptive effect. The specific role of spinal receptors (mGluRs) belonging to groups I and II in intermediating in nociception in behavioral studies was described in rats[18] and in sheep[19] as well as in electrophysiological studies in rats.[20] Intrathecal application of those antagonists in conscious sheep caused inhibition of allodynia provoked mechanically[19] and in rat model of neuropathic pain.[21] There are no detailed data on the role of central receptors of group I (mGluR) in acute intestinal pain, containing both vegetative and emotional, from higher cortex structures, components, and not only spinal receptor or peripheral pain. Hence the objective of the study is to find out the role of mGluRs antagonist infused intracerebroventricularly (*i.c.v.*) in experimental model of intestinal colic (extension of descending colon wall) in conscious sheep. A quanting a functional role of these receptors in central origin nociception may be helpful in an introduction of new analgesics into therapy of acute intestinal pain in ruminants.

MATERIAL AND METHODS

Preparation of animals

Experiment was performed on 6 mature crossbred ewes, Polish merino sheep weighing 35-45 kg B.W., being in *anoestrus* period. Food was withhold 24 hours prior to the experiment. Analgesia was initiated by *i.m.* ketamine (Calypsovet, 20 $mg.kg^{-1}$ B.W., GEDEON RICHTER, Budapest, Hungary) administration and 15 min later *i.v.* infusion of pentobarbital anaesthesia (Vetbutal, 20 $mg{\cdot}kg^{-1}$ B.W., BIOWET, Pulawy Poland) was performed. During unconsciousness a T-shaped silicon cannula (inside diameter of 21 mm), was inserted into the dorsal sac of the rumen, using techniques described previously[22] of all animals. Simultaneously, under the same general anaesthesia/analgesia a permanent stainless steel cannula, 29 mm length and 2 mm in diameter (guide cannula), was inserted into the lateral ventricle (on the left and/or the right side) of the brain, 10 mm above the bregma and five mm laterally from the midline suture using stereotaxic method described by Kania et al.[23] When the animals recovered from general anaesthesia, they were placed in individual boxes, and maintained at an ambient temperature (18 - 20^{o}C), for 10 days to recover. Animals had free access to hay and water, except during the experimental period. The cannulas cared were toileted one time for every experiment.. Experiments were

carried out in fourth stages (groups, each of six animals). Every experiment was performed simultaneously on two unfed animals, which were placed in two individual metabolic cages at one week intervals. Blood was collected 30 min prior the experiment, at 0 time, and 5, 10, 15, 30, 60 and 120 min later (Figure. 1). In the first experimental group, after 60 min control recording of the reticulo-ruminal motility was performed in each animals (n=six) receiving 100 μl of 0.9% NaCl, during one min infusion (20 min after the first blood collection) *via* a silicone catheter (inner cannula - 31 mm in length and 0.5 mm in diameter), into the lateral ventricle of the brain *(i.c.v.);* an then the reticulo-ruminal motility was recorded for 90 min. Venous blood for analysis was collected accordingly to the figure 1. In the second group of animals (n=six), after 60 min recording of reticulo-ruminal contractions each animal received every dose of the drugs (with one week interval; that was 24 individual sheep experiments). After the second collection of the venous blood, the sheep were *i.c.v.* given a one min lasting infusion of 100 μl of L-AP3 (in 0.9% NaCl solution) in a dose of 0.2 mg in the first, 0.4 in the second, 0.8 mg *in toto* in the third week *via* catheter of 0.5 mm in diameter and then the recording was continued for another 90 min. In the third group of animals (n=six), after 30 min of control recording of the reticulo-ruminal motor activity, a rubber balloon a10 cm in length was introduced into the colon *via* the anus and left for 30 min; immediately after the second blood collection (0 time) the balloon was filled with 150 and/or 200 ml of warm water (CD 150 and/or CD 200) and the distension of the descending colon was maintained for five min as a viscero-visceral inhibitory reflex (i.e. descending colonal distension = reticulo-ruminal motor activity inhibition).[22] Then the recording of the reticulo-ruminal contractions was continued for 60-90 min. 10 min before CD each sheep received *i.c.v.* infusion of 100 μl of 0.9% NaCl solution (solvens for L-AP3) (Figure. 1). In the fourth group of animals (n=six) after 30 min of control recording of the reticulo-ruminal contractions a rubber balloon of 10 cm in length was introduced into the colon and 30 min later the animals received the100 μl *i.c.v.* infusion of L-AP3 (in 0.9% NaCl solution) at a dose of 0.2 mg in the first, 0.4 mg in the second, or 0.8 mg *in toto* in the third (i.e. approximately 5.0, 10.0 or 20 $\mu g \cdot kg^{-1}$ B.W.) in the third week. After 10 min since the L-AP3 during one min infusion the descending colon was distended for five min with the balloon containing 150 and/or 200 ml of water (CD 150 and/or CD 200) at the body temperature. After the five min distension was over, the recording was continued for 60-90 min. Experimental procedure lasted six month. The doses of 0.2, 0.4 and 0.8 mg L-AP3 *in toto* were effective in premedication contra CD 150 and CD 200.

Mechanography

The reticulo-ruminal contractions were analysed using the electric tensometric recorder PIT 212 (COMT, Bialystok, Poland). The analysis of mechanograms and calculations of results were performed similarly as in case of electromyographic recording.[23] The frequency of the reticulo-rumen contractions was determined by the number on the mechanograms with 5 min intervals prior and after the colonal distension.

The determination of blood plasma cortisol concentration

The cortisol concentrations were determined by radioimmunoassay (RIA), according to previous experiments.[23] The mean intra and inter assay coefficients of variations for cortisol were 6.1% and 3.5%, respectively. The limit of quantification of the method was of 9.5 pg for a sample of 10 μl (Orion Diagnostica, Espoo). The Δ_{max} concentration for each hormone was the difference between the basal concentration and the highest concentration measured.

The determination of blood plasma catecholamines (CA) concentration

Blood samples were taken from the jugular external vein (according to the procedure described above – Figure. 1). Blood samples were colected into 10 ml test tubes containing reduced glutation (0.05 mM). The plastic tubes were maintained on ice and after centrifugation plasma was stored in -80^{o}C until beginning of the analytical process. The determination of CA concentrations was performed by HPLC with electrochemical detector[24].

The measurement of cardiac and ventilation frequencies

Heart and respiratory rates were determined in all animals by examining the number of heart beats as well as by observing the respiratory thoracic movements using stethoscope for one min. Those measurements were carried out by the same person before each blood test for analysis**.**

The use of chemical substances

In the present experiments the following drugs were used: L-2-Amino-3-phosphonopropionic acid (L-AP3) a nonselective metabotropic glutamate receptor (mGluR) blocker – (USP grade – A154, SIGMA-Aldrich), heparin (Heparinum - POLFA); reduced glutathione (Glutathione, Ethylester, SIGMA-RBI), procaine (2% solution, Polocainum hydrochloricum – POLFA). L-AP3 was dissolved in 0.9% NaCl. The lack of effect of solvent was determined in preliminary experiments. Two days before the planned experiment, under local anaesthesia by *s.c*. injection of 2 ml of 2% procaine (Polocaine hydrochloride, POLFA, Poland), a silicone cannula was inserted into the jugular external vein.

Statistical analysis

Statistical analysis of the results was performed using one way ANOVA. The statistical relevance of the results was determined with a post hoc Tukey-Kramer test. The comparison between the two groups was performed using a *t*-test. The results are presented as a x±SEM. A *P* value less than 0.05 was considered statistically significant in all test. The experiment was performed with accordance to the Principles of Laboratory Animal Care (NIH publication no. 86-23, revised 1985) as well as the specific national laws on protection of animal (National law for animals protection - 1997, Dz. U. 23 XI; Permission of 3rd Local Ethical Commission No 9/2001 issued 11.01.2001). After completion of the experiments all animals were subjected to euthanasia using Morbital (BIOWET, Poland).

Figure 1:

RESULTS AND DISCUSSION

The descending colonal distension upon behavioural symptoms, clinical signs, reticulo-ruminal motility, cortisol and plasma catecholamine concentration was noted. Prior to any manual activity no deviation from normal behaviour was observed in a pair of animals, which were simultaneously placed in separate cages, as long as both motor and behavioural reaction to external stimuli are concerned. The average cardiac frequency was 65.5 and the rate of respiration 18.6·min^{-1}, the number of reticulo-ruminal contractions was 5.5±0.28 c·5min^{-1}. After the insertion of an empty rubber balloon into the colon 30 min prior to CD, no significant changes were observed in animal behaviour or in cardiac action (67.5 beats·min^{-1}), respiration frequency (17.2·min^{-1}) and reticulo-ruminal contractions (5.8±0.35 c·5min^{-1}). There were no significant differences amongst results noticed prior to the insertion of balloon inside the colon. The mechanical distension of the descending colon walls caused highly significant changes in the behaviour, clnical symptoms, reticulo-ruminal motility as well as in plasma cortisol and CA concentrations of animals.

The nonsignificant influence of icv infused saline upon behavioural and clinical symptoms, cardiac and respiratory activity, cortisol and catecholamine blood plasma concentrations.

Infusion of the 100 µl 0.9% NaCl *icv* during one min, 10 min before time 0, in the six animals tested (group I) did not cause any significant ($p \geq 0.05$) changes in behavioural responses (Table 1), clinical symptoms, reticulo-ruminal contractions (±0.25 – 0.5 c·5 min^{-1}; Figure. 2), cortisol (from 1.025±0.05 to 0.916±0.11 ng·ml^{-1}; Figure. 3), epinephrine (from 0.841±0.06 to 0.881±0.04 ng·ml^{-1}; Figure. 4), norepinephrine (from 0.911±0.02 to 0.923±0.01 ng·ml^{-1}; Figure. 5) as well as dopamine (from 0.738±0.07 to 0.845±0.09 ng·ml^{-1}; Figure. 6) of the blood plasma concentrations

in sheep during 120 min observations. A that was in accordance with previous studies.[23]

The significant influence of CD upon general behaviour, clinical symptoms, cortisol as well as plasma catecholamine concentration

The following changes of animal behaviour linked with the action of algesic (nocifensive) factor were observed: an increase in general motricity, bleating, teeth clenching, prostration, wetting, defecation, tachycardia (from 60.3 to 76.2 beats·min^{-1}), hyperventilation (from 16.8 to 24.3 number·min^{-1}) and reticulo-ruminal motility inhibition (from 6.2±1.0 in control to 3.4 c·5 min^{-1} during CD; $p \leq 0.001$). Mechanical distension of the descending colon causes an inhibition of reticulo-rumen contractions count. That was fast effect (few minutes from beginning of distension). The range of contractions during the CD was also reduced to about 25% of quantity observed in control animals. In 75% of the animals a total lack of motility lasting 6 to 10 minutes was observed. A statistically significant inhibition of contractions frequency (average 59.3%) was still observed over 20 minutes following CD termination. The amplitude of contractions during CD was also reduced by 86% comparing to the untreated animals (unpublished data). The descending colonal distension with 150 and 200 ml of water caused a high increase in cortisol (Figure. 3) and CA plasma concentration, in particular of epinephrine, in average of 76.2% during 30 min following CD (increase from 0.781± 0.05 in control to 1.376±0.09 nmole·L^{-1} during CD episode and to 0.880± 0.08 nmol·L^{-1} in 120 min) (Figure. 4). At the same time a statistically significant increase of NE concentration - in average for 15 min by 62% - (from 0.923± 0.01 in the control to 1.570± 0.05 in 15 min) and significant decrease to 0.758±0.04 nmol·L^{-1} 120 min after CD (Figure. 5). This increase was statistically significant up to 15 min of the experiment ($p \leq 0.05$). The concentration of DA increased in average by 90% and lasted for about 10 min (increase from 0.845±0.09 in the control to 1.995±0.21 nmol·L^{-1} 10 min after CD (Figure. 6). This increase was statistically significant during the first 10 min ($p \leq 0.05$).

The significant influence of L-AP3 premedication on behaviour changes, clinical symptoms, reticulo-rumen motility, cortisol and plasma catecholamines level in animals with and nonsignificant without CD

One minute L-AP3 *i.c.v.* infusion in doses 0.2, 0.4 and/or 0.8 mg *in toto*, did not have any significant impact on behaviour and clinical symptoms (Tab. 1), reticulo-rumen contractions number (Figure. 2), cortisol and catecholamines concentration in blood plasma (Figure. Figure. 3-6). The *i.c.v.* infusion of L-AP3 (0.2, 0.4 or 0.8 mg *in toto*) given 10 min prior CD decreased intensity or even prevented appearance of clinical signs of visceral pain caused by CD test (Tab.

1). In sheep without premedication intensive tachycardia was noticed (average from 65 to 78 heart bits·min^{-1}) and in the group of animals receiving L-AP3 the pain caused by CD decreased significantly the heart rate average from 65 to 54 bits·min^{-1}. Respiration frequency was 25 and 15·min^{-1}, respectively. Also the inhibitory effect of CD on frequency of reticulo-rumen motility was significantly lower in the group of animals pretreated with L-AP3 until 10 minutes following CD termination (3.4 and 5.6 c·5min^{-1}, respectively) (Figure. 2). L-AP3 premedication diminish also the increase of plasma cortisol (Figure. 3) and epinephrine concentration caused by visceral pain provoked by CD (Figure. 4). Only a significant increase from 0.90±0.11 to 1.45±0.44 nmol·L^{-1} (+61.1%) during CD lasting and to average 1.68±0.12 nmol·L^{-1} (+86.6%) 10 min later was noticed (differences significant) after premedication by L-AP3 in the high doses (0.8 mg *in toto*). The significant inhibition of NE concentration changes in CD conditions by L-AP3 in the doses of 0.2 and 0.4 mg to 30 min (Figure. 5) as well as DA in 15 min experimentation were observed (Figure. 6). The L-AP3 prevents normal homeostatic process by blocking presynaptic glutamate receptors mGluRs. During the high activity periods, the high EPSPs were observed in neuromuscular plates. Centrally applied L-AP3 in physiological solutions provoked the situation in which brain neurons might not differentiate the glutamate concentration in the synaptic gap, what occurs in the case of the neuromuscular plate[25]. The L-AP3 binding auto-receptors cancels a glutamate release efficiently, breaking the transduction of the action potential. It was confirmed in the conditions by the amplitudes of the EPSPs obtained in the conditions of: a low frequency excitation (0.5 Hz), a high concentration of glutamate and the DL-AP3 addition to a water bath with neuromuscular plate. The results of Ames et al.[25] confirmed current knowledge about the character of group II glutamate receptors. It was shown, that group II metabotropic glutamate receptors (mGluRs) play key role in modulation of the glutamate concentration[15]. Low glutamate concentration in synapse does not excite group II metabotropic glutamate receptors to the inhibition of glutamate releasing. During the high activity of the neuromuscular plate (10 Hz excitation), the neuron decreases the flow rate of glutamate to the synaptic gap. This fact confirms the existence of negative feedback. It may be supposed in our work, that the preventive action of the L-AP3 infused into the cerebral ventricle in the doses of 0.2 and 0.4 mg *in toto* in sheep subjected CD test, was related to the breaking of the noxious signalling in the CNS (so called pain gating). It was followed by the lack of the negative reactions accompanying visceral pain, caused mechanically by the 5 min distension of the descending colon wall with the balloon filled with 150/200 ml of water. The CD caused a strong excitation of hypothalamo-hypophyso-

cortico-adrenal axis (increased cortisol concentration), increased activity of sympatho-medullo-adrenal system (higher plasma concentration of catecholamines) and of motivatio-emotional system causing a lot of behavioural changes. The following clinical symptoms of this stimulation were observed: tachycardia, hyperventilation, inhibition of reticulo-rumen motor function (attenuation of viscero-visceral inhibitory reflex), defecation and/or urination, looking to the sides, escaping reflex from the experimental cage, and other behavioural syndromes (Tab. 1). On the base of the biochemical and behavioural analyses, mechanical pain stimulus appeared to be stressogenic and caused general physical stress in experimental animals[26]. Considering an action of L-AP3, as a potential antagonist of the CNS group I metabotropic receptors and its antynociceptive action, the analysis of the CD effect after 10 minutes from the premedication was performed. The L- isomeric form of the AP3 was applied into lateral cerebral ventricle (*i.c.v.*) in the doses of 0.2, 0.4 and 0.8 mg/animal during one min (see material and methods). The premedication with this antagonist of the group I metabotropic glutamate receptors (mGluRs) cancelled, prevented or inhibited the influence of noxious factor (CD). The L-diffracted AP3 isomer, infused *i.c.v.* in the doses of 0.2 and 0.4 mg *in toto* at 10 min before the colon distension with water (150/200 ml) during five min, significantly decreased the release of cortisol and catecholamines (stress hormones), diminished or weakened emotional reactions of animals related to the excitation of intracerebral motivational structures i.e. limbic system, striatum, globus pallidus, thalamus, hypothalamus and medial forebrain bundle[27]. Peripheral damaging stimuli excite specific receptors on nociceptive afferent neurons, evoking the action potentials and releasing of glutamate and neuromodulatory peptides from axon endings in spinal cord. Afferent neuron endings form terminal synaptic bulbs with neurons of dorsal horns of spinal cord and these cells project to the perception cortex centres. Glutamate released from the endings of afferent neurons affect neurons of dorsal horns by excitation of two groups of receptors: glutamate ionotropic receptors (iGluRs) and glutamate metabotropic receptors (mGluRs). The iGluRs are ligand-gated ion channels: NMDA, AMPA and cainic receptors. These receptors participate in quick synaptic transmission from afferent neurons to dorsal spinal horn and to superior sensory centres in brain. Receptors mGluRs include whole family of the receptors related through proteins G to different intracellular systems of the secondary messengers. These receptors mediate in neuromodulating action of glutamate.[28] Glutamate receptors are present in both, peripheral and central nervous systems. Fisher et al.[21] found the localisations of mGluRs in spinal cord and brain. Bhave et al.[4], Carlton and Neugebauer[5], Walker et al.[6] stated that the mGluRs localised already in the first ascending neurons may participate in

the modulation of pain. Kirchgessner[29] in studies with Guinea pigs and rats revealed the presence of glutamate receptors of both types, ionotropic and metabotropic, particularly of group I mGluRs in ganglions of mesenteric plexuses and intestinal epithelium. The author claimed that these receptors play an important role in regulation of intestinal peristaltics and in gastrointestinal secretion. Hence the application of the antagonists of metabotropic and/or ionotropic receptors as potential analgesic drugs may work in peripheral as well as in central nervous system. The results of analgesic action of the L-AP3, in mechanically evoked pain test (descending colon distension), which occurred as the non-specific antagonist of the group I mGluR receptors, confirm literature data obtained after application of specific and non-specific antagonists of mGluR, including group I, in chronic and acute pain tests in rodents. There is no doubt, that plasticity of synapses mediating in nociceptive process needs the activation of the NMDA receptors. However in many cases this plasticity needs also modulation by mGluR.[30] As it was emphasised in the introduction, many subtypes of mGluRs are present in noxious tracts and studies performed in recent years determined the role of the mGluRs in noxious transduction and synaptic plasticity. The application of the mGluR ligands into brain ventricle in sheep, caused that these molecules bind firmly to the specific group I mGluRs preventing in this way neurons from depolarisation induced by specific ligand i.e. glutamate. Glutamate, as a specific agonist of the mGluRs increases ability and rate of transduction of noxious stimuli and intensifies sensitivity of the pain perception centres including spinal cord, thalamus and brain prefrontal cortex. The prior *i.c.v.* premedication with drug of the opposite action – antagonistic to glutamate – resulted in its firm binding to the group I mGluRs. Even massive release of glutamate resulting from CD did not overcome the blockade of stimulus transmission to the central structures of pain transduction. Moreover this blockade did not allow, at least periodically, to elicit the analysed cascade of biochemical (cortisol, catecholamines) and behavioural changes which might be a consequence/ symptoms of noxious stimulus. The L-AP3 reversed the sensitivity and prevented the development of the central perceptivity caused by the joint inflammation.[31] In our study, we extrapolated doses applied peripherally in rodents, considering the amount of active substances in organic fluid. Such calculated doses of the AP3 were 10-fold decreased (*i.m.* injection versus *i.c.v.* injection), respectively and applied in different amounts accordingly to the pharmacological procedures. Doses of tested substance (L-AP3) were not too big for sheep, because later no adverse effects were observed. The antagonists of the NMDA receptors were used in chronic pain treatment after traumas[32]. However this author recognised a significant adverse effects after the application of non-specific antagonists for

particular subgroups of the NMDA receptors, what to the great extent caused the critical opinions on the role of the NMDA receptors in the normal excitatory synaptic transduction in nervous system. Those adverse effects included fatigue, dizziness, psychoses, hyperactivity, and in the case of the high doses of the NMDA antagonists: memory losses and damage of neurons[33]. Obtained data demonstrated that the development and maintenance of the visceral pain symptoms of the CD is dependent on activation of $mGluR_1$ in the CNS and that these receptors play a crucial role in modulating experimental acute visceral pain. The group I of the mGluR antagonist prevent behavioral, clinical and neuroendocrine symptoms of visceral pain. They can be possibly used in cases of trauma or as an analgesic in acute/chronic visceral pain, especially, in combination with opioid agonists, without adverse effects inseparably related to the application of the antagonists of the NMDA receptors.

Table.1. The effect of colonal distension (CD) on the reticulo-ruminal activity (inhibition in %/5 min in comparison to the control values) and behavioural symptoms (number/5 min) in sheep before and after *icv* L-AP3 pretreatment at a dose of 0.4 mg *in toto* (i.e. 10 μg·kg $^{-1}$ B.W., n=6)

Accompanying symptomps	-5 - 0		0 - 5		5 – 10		10 – 15		25 - 30		55– 60		120 min	
	CD	AP3 +CD	CD	AP3+CD	CD	AP3 + CD	CD	AP3 +CD	CD	AP +CD	CD	AP+ CD	CD	AP3 +CD
Inhibition reticulo-ruminal activity	-	-	4+ 80	+ 19	4+80	+ 11	3+ 48	+ 15	2+ 38	+ 22	2+ 45	-	2+ 30	-
Looking around	-	-	3+	±	3+	±	2+	-	+	-	-	-	-	-
Defecation and/or urinating	-	-	+	-	+	-	+	±	-	+	-	-	-	-
Head movements	-	-	3+	-	2+	±	-	-	-	+	-	-	-	-
Streching	-	-	+	-	+		-	-	-	+	-	-	-	-
Grinding	-	-	+	±	-	+	-	±	-	-	-	-	-	-
Streching out	-	-	2+	-	+	-	-	±	-	-	-	-	-	-
Bleating	-	-	+	-	2+	-	+	-	-	-	-	-	-	-
Tachycardia	-	-	2+	-	2+	±	2+	±	±	±	3+	±	3+	-
Hyperventilation	-	-	-	-	+	±	+	±	+	-	+	-	+	-

Abbreviations: - no reaction, ± occasional, 2+ quite frequently, 3+ frequently,4+ very frequently

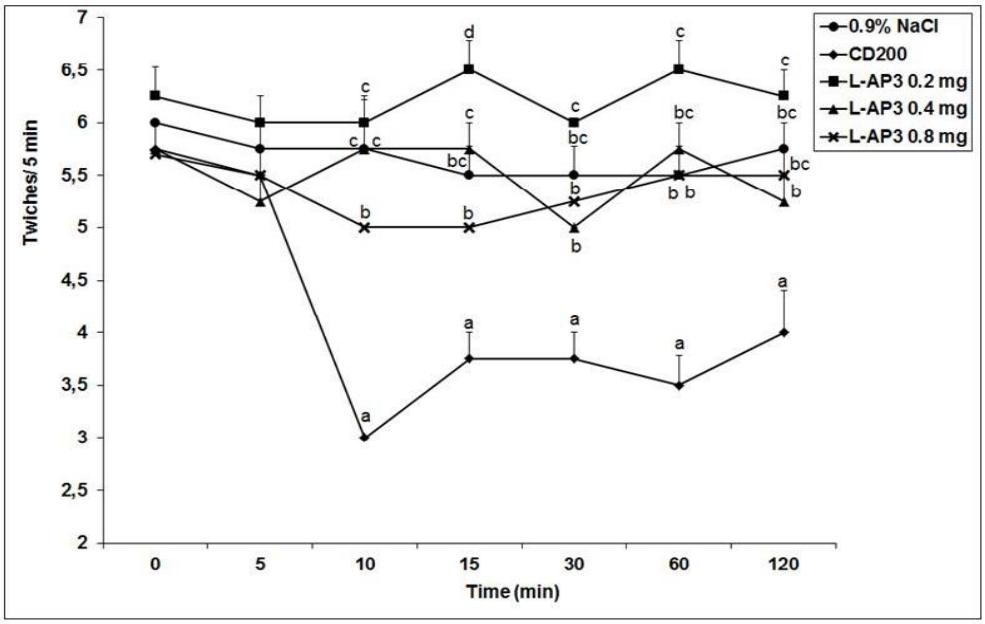

Figure 2: Influence of *i.c.v.* infusions of different L-AP3 doses (0.2, 0.4 and/or 0.8 mg/animal) per number of ruminal contractions (c/5min) in sheep in comparison with the group with 0.9% NaCl and CD200 (x ±SEM, n=6, p≤0,05), [a, b, c, d]- different letters indicate statistically significant distinctions when p≤0.05.

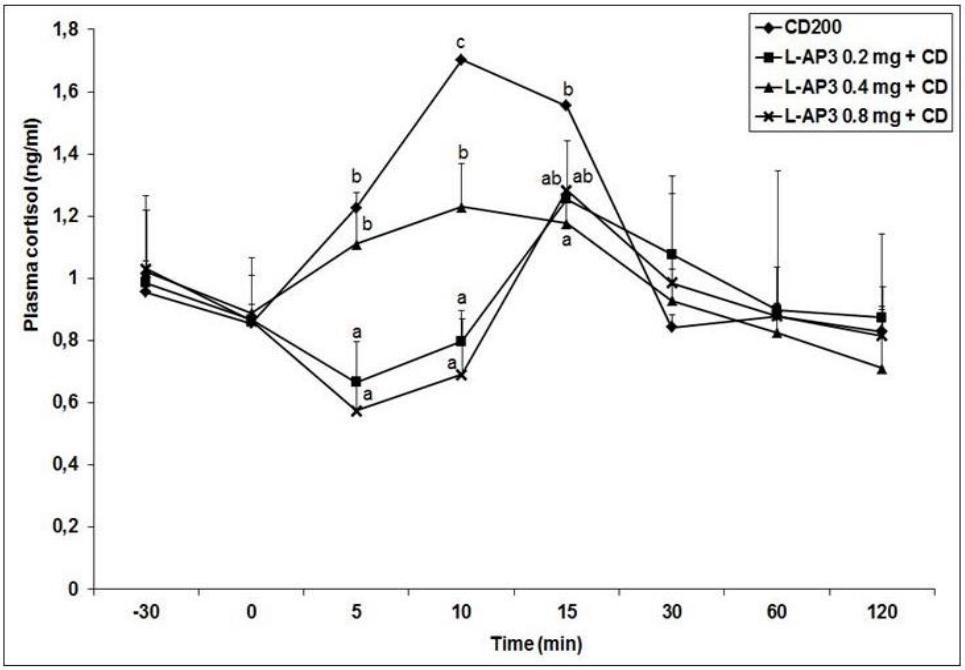

Figure 3: Comparative analysis of premedication influence with *i.c.v.* L-AP3 (in doses of 0.2, 0.4 and/or 0.8 mg/animal) and CD on plasma concentration of cortisol in sheep in comparison with CD200 (x ±SEM, n=6, p≤0.05) , [a, b, c]- different letters indicate statistically significant distinctions when p≤0.05.

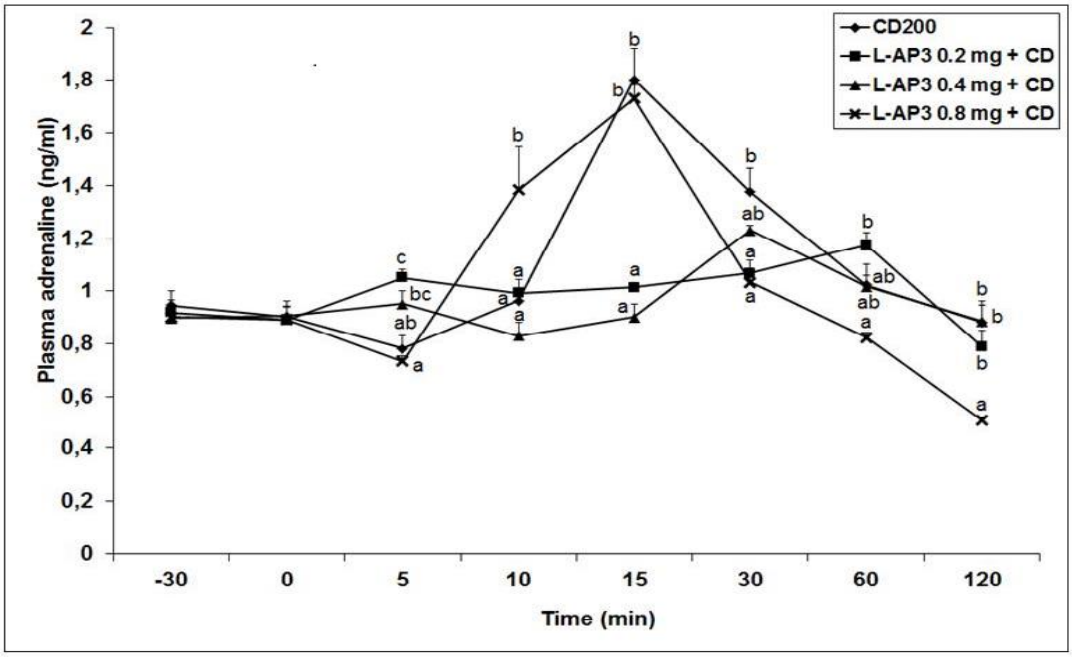

Figure 4: Comparative analysis of colon distension and different doses of L-AP3 influence on plasma concentration of E in comparison with CD200 (x±SEM, n=6, p≤0,05), [a, b, c] - different letters indicate statistically significant distinctions when p≤0,05.

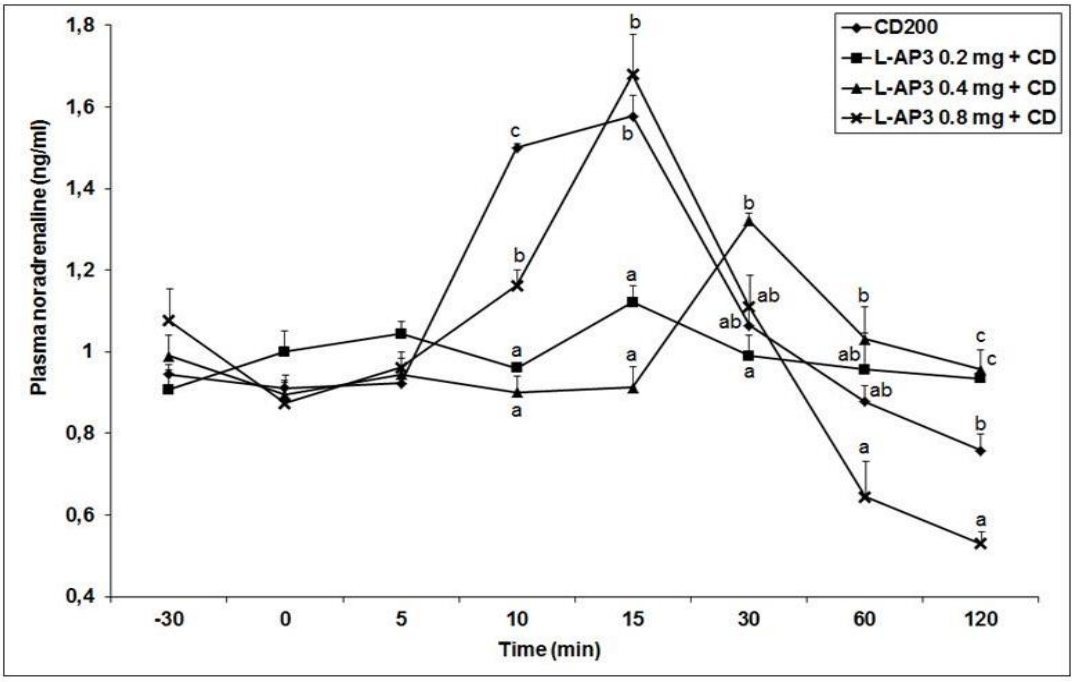

Figure 5: Comparative analysis of colon distension and premedication with different L-AP3 doses influence on plasma concentration of NE in comparison with CD200 (x ±SEM, n=6, p≤0,05), [a, b, c] - different letters indicate statistically significant distinctions when p≤0,05.

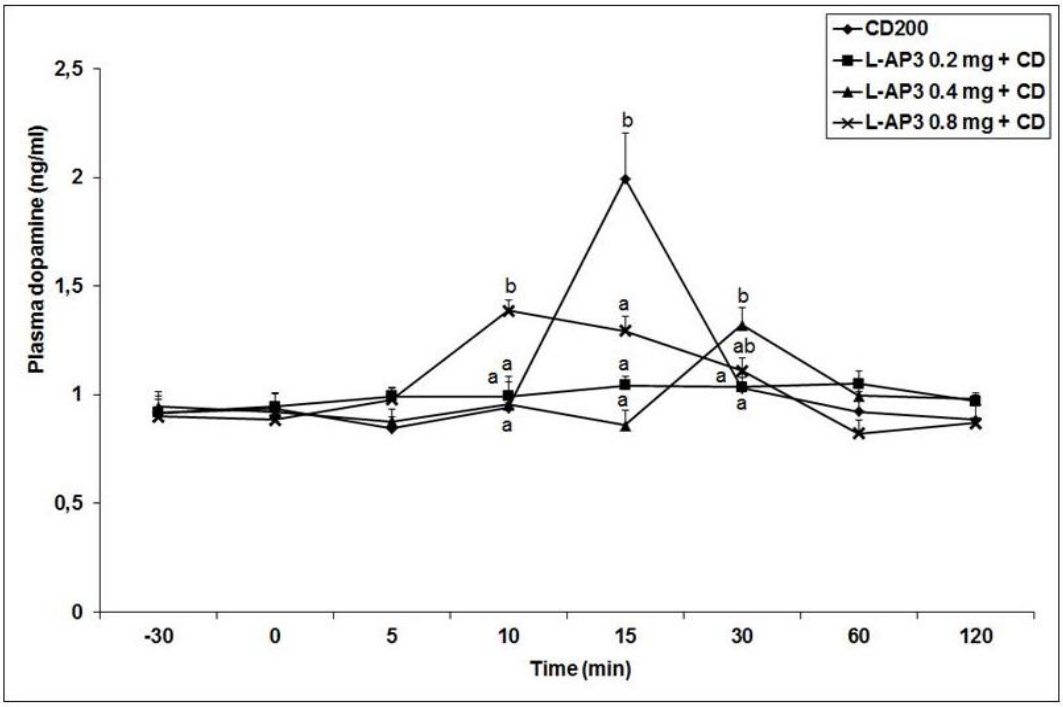

Figure 6: Colon distension and different L-AP3 doses influence on plasma concentration of DA (x ±SEM, n=6, p≤0.05), [a, b, c] - different letters indicate statistically significant distinctions when p≤0.05.

CONCLUSION

REFERENCES

1. Pin J.P., Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neurpharmacol. 1995; 34: 1-26.
2. Cartmell J., Schoepp D.D. Regulation of neurotransmitter release by metabotropic glutamate receptors. J. Neurochem. 2000; 75: 889-907.
3. Giessel A.J., Sabatini B.L. Boosting of synaptic potentials and spine Ca transients by the peptide toxin SNX-482 requires alpha-1E-encoded voltage-gated Ca channels. PLOS One. A Peer-Revieved, Open Acces Journal 2011;6(6):e20939. Epub 2011.
4. Bhave G., Karim F., Carlton S.M. et al. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. Nat. Neurosci. 2001; 4: 417-423.
5. Carlton S.M., Neugebauer V. Peripheral metabotropic glutamate receptors as drug target for pain relief. Expert Opin. Ther. Targets 2002; 6: 349-361.
6. Walker K., Bowes M., Panesar M. et al. Metabotropic glutamate receptor subtype 5 (mGluR5) and nociceptive function. I. Selective blockade of mGluR5 receptors in models of acute, persistent and chronic pain. Neuropharmacol. 2001; 40: 1-9.

7. Zhang G.H., Min S.S., Lee S.K. et al. Intraarticular pretreatment with ketamine and memantine could prevent arthritic pain: relevance to the decrease of spinal c-fos expression in rats. Anesth. Analg. 2004; 99: 152-158.
8. Azkue J.J., Mateos J.M., Elezgari I. et al. The metabotropic glutamate receptor subtype mGluR2/3 is located at extrasynaptic loci in rat spinal dorsal horn synapses. Neurosci. Lett. 2000; 287: 236-238.
9. Lee K.S., Kim J., Yoon Y.W. et al. The peripheral role of group I metabotropic glutamate receptors on nociceptive behaviors in rats with knee joint inflammation. Neurosci. Lett. 2007; 416: 123-127.
10. Sillevis S.P., Kinoshita A., de Leeuw B. et al. Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. New. Eng. J. Med. 2000; 342: 21-27.
11. Tamaru Y., Namura S., Mizuno N. et al. Distribution of metabotropic glutamate receptor mGluR3 in the mouse CNS: Differential localisation relative to pre- and postsynaptic sites. Neurosci. 2001; 106 481-503.
12. Bradley S.R., Standaert D.G., Rhodes K.J. et al. Immunohistochemical localisation of subtype 4a metabotropic glutamate receptors in the rat and mouse basal gandlia. J. Com. Neurol. 1999; 4407: 33-46.
13. Romano C., Sesma M.A., Mc Donald C.T. et al. Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. J. Comp. Neurol. 1995; 355: 455-469.
14. Duvoisin R.M., Zhang C., Ramonell K. A novel metabotropic glutamate receptor expressed in the retina and olfactory bulb.J. Nneurosci. 1995; 15: 3075-3083.
15. Schoepp D.D. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J. Pharmacol. Exp. Therap. 2001; 299: 12-20.
16. Azkue J.J., Knopfel T., Kuhn R. et al. Distribution of the metabotropic glutamate recepptor subtype mGluR5 i rat midbrain periaqueductal gray and relationship with spinofugal afferents. Neurosci. Lett. 1997; 228: 1-4.
17. Li J., McRoberts J.A., Ennes H.S. et al. Experimental colitis modulates the functional properties of NMDA receptors in dorsal root ganglia neurons. Am. J. Physiol. -Gastrointest. Liver Physiol. 2006; 291: G219-G228.
18. Fundytus M.E., Yashpal K., Chabot J.G. et al. Knockdown of spinal metabotropic glutamate receptor 1 (mGluR1) alleviates pain and restores opioid efficacy after nnerve injury in rats. Br. J. Pharmacol. 2001; 132: 354-367.

19. Dolan S., Nolan A.M. Behavioral evidence supporting a differential role for spinal group I and II metabotropic glutamate receptors in inflammatory hyperalgesia in sheep. Neuropharmacol. 2002; 43: 319-326.
20. Budai D., Larson A.A. The involvement of metabotropic glutamate receptors in sensory transmission in dorsal horn of the rat spinal cord. Neurosci. 1998; 83: 571-580.
21. Fisher K., Fundytus M.E., Cahil C.M. et al. Intrathecal administration of mGluR compound, (S)-4CPG, attenuates hyperalgesia and allodynia associated with sciatic nerve constriction injury in rats. Pain 1998; 77: 59-66.
22. Brikas P., Kania B.F., Fioramonti J., Buéno L. Central and peripheral serotonergic influences on viscerovisceral inhibitory reflex during duodenal distension in sheep. Dig. Dis. Sci. 1993; 38: 1079-1086.
23. Kania B.F., Sutiak V. Influence of centrally administered Diltiazem on behavioural responses, clinical symptoms, reticulo-ruminal contractions and plasma catecholamine level after experimentally induced duodenal distension in sheep. Res. Vet. Sci. 2011; 90: 291-297.
24. Wang Y., Fice D.S., Yeung P.K.F. A simple high-performance liquid chromatography assay for simultaneous determination of plasma norepinephrine, epinephrine, dopamine and 3,4-dihroxyphenyl acetic acid. J. Pharmac. Biomed. Anal. 1999; 21: 519-525.
25. Ames P., Heto N., Ramdial R. L-2-Amino-phosphonopropionic acid modulates the release glutamate by blocking presynaptic group II mGlu receptors at neuromuscular junction in Procambarus clarkii. Pioneering Neurosci. 2002; 3: 21-26.
26. Kania B.F., Wronska-Fortuna D., Romanowicz K. et al. Autonomic system and hypothalamo-hypophyso-cortico-surrenalienne reactions in sheep during duodenal distension (pol.). Medycyna Wet. 2001; 57: 338-341.
27. Kania B.F., Siwecka B. (2003) Pharmacological and therapeutical aspects of pain attenuation in animals (pol.). Magazyn Weterynaryjny 2003; 12: 63-66.
28. Varney M.A., Gereau I.V. Metabotropic glutamate receptor involvement in model of acute and persistent pain: prospects for the development on novel analgesics. Curr. Drug Targets-CNS&Neurol. Disorders 2002; 1: 283-296.
29. Kirchgessner A.L. Glutamate in the enteric nervous system. Curr. Opin. Pharmacol. 2001; 1: 591-596.
30. Neugebauer V. Metabotropic glutamate receptors - important modulators of nociception and pain behavior. Pain 2002; 98: 1-8.

31. Neugebauer V., Lücke T., Schaible H.G. Requirement of metabotropic glutamate receptors for the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons. Eur. J. Pharmacol. 1994; 6: 1179-1186.
32. Hewitt D.J. The use of NMDA-receptor antagonists in the treatment of chronic pain. Clin. J. Pain 2000; 16: 73-79..
33. Eide K., Stubhaug A., Oye I. et al. Continuous subcutaneous administration of the N-methyl-D-aspartic acid (NMDA) receptor antagonist ketamine in the tretment of post-herpetic neuralgia. Pain 1995; 61: 221-228.

.