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Research Article

INFLUENCE OF GROUP I mGluR's ANTAGONIST ON THE AUTONOMIC RESPONSES TO COLONAL PAIN IN SHEEP

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ABSTRACT

The present study examined contribution of group I metabotropic glutamate receptors mGluRs to development and maintenance of changes in cortisol and catecholamines blood concentrations caused by visceral pain produced by colonal distension (CD) in sheep. Intracerebroventricular (*icv*) administration of the group I mGluRs non-selective antagonist DL-2-Amino-3-phosphonopropionic acid (DL-AP3; 4.0 8.0 and/or 12.0 mg *in toto*), 10 min before, blocked development of vegetative visceral pain symptoms and neuroendocrinological changes in the blood of sheep. This data demonstrated that development and maintenance of visceral pain symptoms of CD is dependent on activation of group I mGluRs in central nervous system (CNS) and that these receptors play a crucial role in modulating of acute colonal pain in sheep.

Keywords: DL-2-amino-3-phosphonopropionic acid, colonal distension, behavior, catecholamines, cortisol

INTRODUCTION

Metabotropic glutamate receptor's are a very complex molecular for major excitatory amino acids neurotransmitters (glutamic acid - glutamate, aspartic acid – aspartate, glycine) bounds. They are composed with two superfamily: on ligand gated ion channels (inotropic-NMDA) composed with three different class (NMDA, AMPA, kainic receptors) and G-protein coupled metabotropic glutamate receptors family (mGluRs) divided into three distinct groups and eight subgroups. Group I – including $mGluR_{1\ and\ 5}$, group II – $mGluR_{2\ and\ 3}$ and group III – $mGluR_{4,\ 6,\ 7\ and\ 8}$. Group I mGluRs with subclass R_1 (inreasing Ca^{2+}) and R_5 (activation of Na^+ channels) stimulating activity of phospholipases *via* synthesis of IP_3 and DAG as a second messengers. *Via* modulation of channels K^+ activity it can cause increase or decrease postsynaptic cell excitability. Group I mGluRs were recognized as slow acting excitatory, inreasing conductance, causing more glutamate to be released from the presynaptic endings, but they also increase inhibitory postsynaptic potentials, or IPSP's[1]. They are primarily localized as a postsynaptic in brain structures (cerebral cortex, dorsal and ventral striatum, septal area, hippocampus, dorsal horn of the spinal cord, enteric nervous system)[2,3,4], but they can also inhibit glutamate releasing from presynaptic endings as a autoreceptors and are widely localized along pain neuraxis and considered to take part in transmission of analgesic activity[5], including sensory neurons projecting to CNS[6]. ICV infusion of MPEP, which is mGluR5 antagonist, reduces mechanosensitivity of ferret tension receptors activated by gastric distension[7]. Several behavioural studies have demonstrated that $mGluR_5$ in the dorsal horn play a crucial role in visceral inflammatory, postsurgical and neuropathic pain and hyperalgesia[8,9].

DL-AP3 racemate, as a phosphono analog of aspartate, is a antagonist of trans-ACPD-activated phoshonoinositide hydrolysis. It is shown that L-AP3 did not decrease nociceptive symptoms caused by formalin injection[10]. There was also shown only weak antagonistic activity against mGluRs group I[11]. The effects of mGluR antagonists, however, on nociceptive symptoms depends on models in which they are tested. The aim of this study was to assess the efficacy of DL-AP3 racemate after *icv* infusion of different doses in cortisolemy and catecholaminemy caused by colonic distension in small ruminants.

MATERIALS AND METHODS

Experiments were carried out in 4 stages (groups, each of 6 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.

In the first experimental group, after 60 min control each animal (n=6) receiving 100 µl of 0.9% NaCl, during 1 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 (*icv*). Venous blood for analysis was collected accordingly to above.

In the second group of animals (n=6), after 60 min control each animal received every dose of the drugs (with one week interval). After the second collection of the venous blood the sheep were given *icv* in 1 min lasting infusion of 100 µl of racemate DL-AP3 (in 0.9% NaCl solution) in a dose of 4.0 mg in the first week, 8.0 in the second one, 12.0 mg *in toto* in the third week *via* catheter of 0.5 mm in diameter and then the experiment was continued for another 90 min.

In the third group of animals (n=6), after 30 min of control a rubber balloon 10 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 colon was maintained for 5 min. 10 min before CD each sheep received *icv* infusion of 100 µl of 0.9% NaCl solution (solvens for DL-AP3).

In the fourth group of animals (n=6) after 30 min of control a rubber balloon of 10 cm in length was introduced into the colon and 30 min later the animals received the100 µl *icv* infusion of DL-AP3 (in 0.9% NaCl solution) at a dose of 4.0 mg in the first, 8.0 mg in the second one or 12.0 mg *in toto* in the third week. After 10 min since the DL-AP3 was dosed during 1 min infusion the colon was distended for 5 min by means of the balloon containing 150 and/or 200 ml of water (CD 150 and/or CD 200) at the body temperature. After 5 min, since distension was over, the experiment was continued for 60-90 min.

The cortisol concentrations were determined by radioimmunoassay (RIA) according to previous experiments[12]. The determination of CA concentrations was performed by HPLC with electrochemical detector.[13]

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 results are presented as x±SEM. *P* value less than 0.05 was considered statistically significant ($P < 0.05$) in all test.

The experiment was performed with accordance to 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).

RESULTS

The influence of 0.9% NaCl *icv* infusion upon cortisol and catecholamine concentrations

Intraventricular infusion of 100 µl of 0.9% NaCl (group I) during the first minute and 10 minutes before 0 time did not cause significant changes in plasma cortisol (from 1.127±0.09 to 0.946±0.09 ng·ml^{-1}), epinephrine (from 0.805±0.04 to 0.923±0.04 ng·ml^{-1}), norepinephine (from 0.900±0.04 to 0.943±0.03 ng·ml^{-1}) and DA concentrations (from 0.955±0.05 to 0.898±0.03 ng·ml^{-1}) in sheep during 120 min of the observation. These results confirm experimental data obtained earlier after 5 min episode of duodenal wall distension (Kania and Sutiak 2011).

The influence of CD upon as plasma catecholamine concentration

The colonal distension with 150 and 200 ml of water caused high increase in cortisol plasma concentration (from 0.974±0.05 to 1,446±0.05 ng·ml^{-1}; $p < 0.05$- Fig. 1) and also CA, particularly of epinephrine (from 0.888±0.02 in control to 1.270±0.03 ng·ml^{-1} for 30 min after termination of CD and to 1.014±0.02 ng·ml^{-1} in 60 min after CD, on average of 26.3% during 115 min following CD (Fig. 2). At the same time statistically significant increase of NE concentration - on average for 115 min by 22.3% - (from 0.951± 0.01 in the control to 1.214± 0.02 ng·ml^{-1} in 15 min – Fig. 3). This increase was statistical significant during 15 min following CD ($p \leq 0.05$). The concentration of DA increased on average by 24.3% and lasted for about first 15 min of the experiment (increase from 0.904±0.02 in the control to 1.124±0.02 ng·ml^{-1} 10 min after CD - Fig. 4). This increase was statistically significant during the first 15 min ($p \leq 0.05$).

The influence of DL-AP3 premedication on cortisol and plasma catecholamines level in animals with/without CD

1 min racemate DL-AP3 *icv* infusion in the doses of 4.0, 8.0 and/ or 12 mg *in toto*, did not have any significant impact on cortisol and catecholamines concentration in blood plasma. DL-AP3 premedication also diminishes the increase of plasma cortisol, E, NE and DA concentrations caused by visceral pain provoked by colonal distension. Premedication with racemate DL-AP3 in the doses of 4.0, 8.0 and/or 12.0 mg *in toto* prevented from significant increase of cortisol concentration by considerable decreasing from 1.067±0.02 in CD group to 0.791±0.03 ng·ml^{-1} in 15 min following CD episode group (Fig. 1). In 10th min of experiment plasma concentrations of cortisol in the group of animals with CD were about 60% higher than those in the group of animals premedicated with DL-AP3 in the doses of 4.0, 8.0 and/or 12.0 mg *in toto* ($p \leq 0.05$). In 15th min after finishing CD, in animals premedicated with 12.0 mg DL-AP3 plasma cortisol concentration was significantly decreased in comparison with its also diminished concentrations after *icv* infusion of drug in a dose of 4.0 mg *in toto* and with relation to the group with CD episode. The only case when significantly higher plasma cortisol concentration was stated in comparison with the groups with DL-AP3 premedication in the dose of 12.0 mg *in toto* as well as with animals with CD episode was ascertained only in 30th min after CD finishing in animals premedicated with 4 mg DL-AP3.

5 min CD episode alone increased plasma E concentrations from 0.888±0.02 to 1.270 ng·ml^{-1} in 15th min of experiment. Intracerebroventricular premedication with racemate substance DL-AP3 in the doses of 4.0, 8.0 and 12.0 mg *in toto* 10 min before 5 min CD episode prevented from significant increase of concentration as it kept up control concentrations level through all the time of experiment (Fig. 2).

Mean decrease of plasma E concentration in DL-AP3 premedication was about 55% ($p \leq 0.05$) and it during for 30 min after finishing CD episode. In remaining time ranges these concentrations in all tested groups of experimental animals were approximate ($p \leq 0.05$).

DL-AP3 applied *icv* in the doses of 4.0, 8.0 and/or 12.0 mg *in toto* caused statistically significant decrease of plasma NE concentration for 30-120 min. It remained particularly long after applying the highest DL-AP3 dose (from 1.062±0.03 to 0.815±0.04 ng·ml^{-1} in 120th min of the observation). In 10th min after DL-AP3 premedication increasing concentration through 5 min of CD was by 60% lower in comparison with the group of animals with CD, exclusively. In 120th min of experiment plasma NE concentration in animals from CD group was statistically lower than in groups premedicated with DL-AP3 in the doses of 4.0 and 8.0 mg *in toto* before CD episode (0.859±0.02 in the CD group and 1.020±0.04 and 0.956±0.04 in groups with DL-AP3 in the doses of 4.0 and 8.0 mg *in toto*). Similar plasma NE concentrations were stated in 120 min of the obserwation in CD group and also in the one premedicated with DL-AP3 in the dose of 12.0 mg *in toto* (0.859±0.02 and 0.815±0.04 ng·ml^{-1}, respectively) (Fig. 3).

Intraventricular infusion of DL-AP3 in the doses of 4.0, 8.0 and/or 12.0 mg *in toto* did not influence significantly on plasma DA concentration (0.926±0.04 in control group, 0.936±0.03 ng·ml^{-1} in 120th min after infusion of *icv* DL-AP3 in a dose of 4.0 mg *in toto*) (Fig. 4).

Colonal wall distension increased significantly plasma DA concentration for 15 min after episode ending (from 0.904±0.02 to 1.124±0.02 ng·ml^{-1}). DL-AP3 premedication in the doses of 4.0, 8.0 and/or 12.0 mg *in toto* 10 min before 5 min CD episode decreased significantly plasma DA concentration in comparison with concentrations in the group with the same CD episode (respectively from 1.045±0.04 as well as 1.986±0.02 ng·ml^{-1}) especially intensively in 15th min of the experiment. In 30th min of the experiment significantly higher plasma DA concentrations in animals premedicated with DL-AP3 in a dose 4.0 mg *in toto* with relation to the group of animals with CD episode were stated (Fig. 4).

DISCUSSION

Obtained results interchangeably point at inhibition of pain sense releasing by centrally used DL-AP3 racemate as a non-specific antagonist of metabotropic glutamate receptor mGluR I group. Minute intracerebroventricular infusion of this drug in sheep diminished and/or prevented from vegetative symptoms neuroendocrine (increase of plasma cortisol and catecholamines concentration) of pain in this study or like changes in behavioural (urinations, defecations, gnawing, moaning, will of escaping from an experimental cage) and clinical symptoms (reticulo-ruminal motility inhibition, tachycardia, hiperventilation) in earlier study[14].

Physiopharmacology of visceral pain was elaborated on a model of mechanical nocifensive impuls emitted by duodenum and/or colon distension (stretch and tension) in rodents[15]. Personal results indicate interchangeably that stretching of an intestine wall is correlated with contraction amplitude of both duodenum and colon. It is also an important impuls for intestines contractility. In contractility response of mice intestine wall to mechanical stimulus nerve regulation is implicate[15].

Distension of a colon wall, similarly to earlier duodenum investigations[12], caused each time significant retardation of viscero-visceral inhibitory reflex which was probably caused by sympatho-adrenal system stimulation and adrenal CA release to circulatory system and also significant increase of plasma cortisol concentration (stimulation of hypothalamo-pituitary-cortico-adrenal axis). The action of mechanical pain stimulus in the intestine proved to be strong general stressor which released classical defensive reactions of the organism. Such reaction was prevented by previous (10 min befor CD) intracerebroventricular premedication with DL-AP3 racemate in 3 different doses. This premedication also prevented from tachycardia, hyperventilation as well as behavioural symptoms of intestinal pain demeanour[14]. It proves that DL-AP3 can not only reveal its peripheral actions but also central ones, mainly by inhibition of metabotropic glutamate receptors mGluR activity in motivational structures and nociceptive afferent pathways that transmit mechanical impulses, such as distension of intestines walls, from periphery to higher structures of nervous system.

Kyloh and co-workers[16] identified out tracts of visceral pain by means of activity of mechanical nocifensive factor which was colorectal acute noxious distension in mice. It can be presumed that irritable bowel syndrome in man and connected with it visceral pain is probably evoked by colorectal distension. Extrinsic afferent nerve pathways that receive impulses and transmit visceral pain from colorectum to the spinal cord are still unknown. On the basis of viscero-somatic reaction in mice, the fact that visceral pain evoked by terminal colorectum segment distension, which was released by damaged mechanical impuls, is conducted through rectal/pelvic nrve to the spinal cord, with cell bodies of which are localised mainly in lumbo-sacral part of the spinal cord, was stated[16].

In mammals there are two distinct spinal afferent nerve tracts that can potentially transmit sensory information from rectum and (sigmoid) distal colon to the spinal cord. They are known as lumbar colonic nerves (LCN)/lumbar visceral ones and sacral colo-rectal ones/pelvic nerves.[17] There was not stated interchangeably up till now which of these pathways is more important in the detection and transmission of visceral pain from colon and/or rectum. Latest results of Feng et al.[18] revealed that in mice these two separate sensory nerve tracts are distinguished by presence of at least 5 different classes of afferent fibres every of which reacts selectively and independently at activity of various impulses. In principle, there is not certain whether colo-rectal pelvic pathway is first of all nerve tract with low excitability treshold which reacts at mechanical stimulation of small intensity whereas lumbar splanchnic pathway is mainly the one that reacts at impulses of high threshold which carrying only small amount (10%) of stretch afferent fibres sensitive to distension in mouse[18].

Presence of metabotropic glutamate receptors in dorsal horns of the spinal cord neurons evoke that intracerebroventricular

intrathecal appliction[19] of specific antagonists for $mGluR_1$ receptors reveals analgesic activity both in rodents and large mammals. Knockdown of spinal $mGluR_1$ metabotropic glutamate receptors alleviates pain and restores opioid efficacy after nerve injury in rats[20]. Recently published results prove that mice which were deletions of endothelin-3 (ET-3 gene) in rectum model of Hirschsprung's disease loss selectively the ability to feel visceral pain.[21] Qi et al.[22] suggest special participation of voltage gated Na^+ and Ca^{2+} channels in visceral pain feeling whereas application of these canals antagonists can contribute to new possibilities in visceral pain therapy.

Results of presented research interchangeably indicate that used centrally non-specific group I mGlur's receptor antagonist – which is racemic form of DL-AP3 – inhibit transmission of nocifensive impuls evoked by 5 min mechanical distension of colonal wall in sheep. It also prevents from cascade of behavioural, clinical and neuroendocrinial phenomena released in acute visceral pain by mechanical factor. DL-AP3 can be recommended in alleviating of intestinal colic symptoms in sheep provided that similar effects after peripheral usage of that racemate will be confirmed.

CONCLUSION

DL-AP3, nonspeciific group I mGluR's antagonist centrally infused attenuate transmission of nocifensive impulses by distension of colonal descending wall provoked in sheep. It also prevents cascade of behavioral, clinical and endocrinial changes in acute visceral pain.

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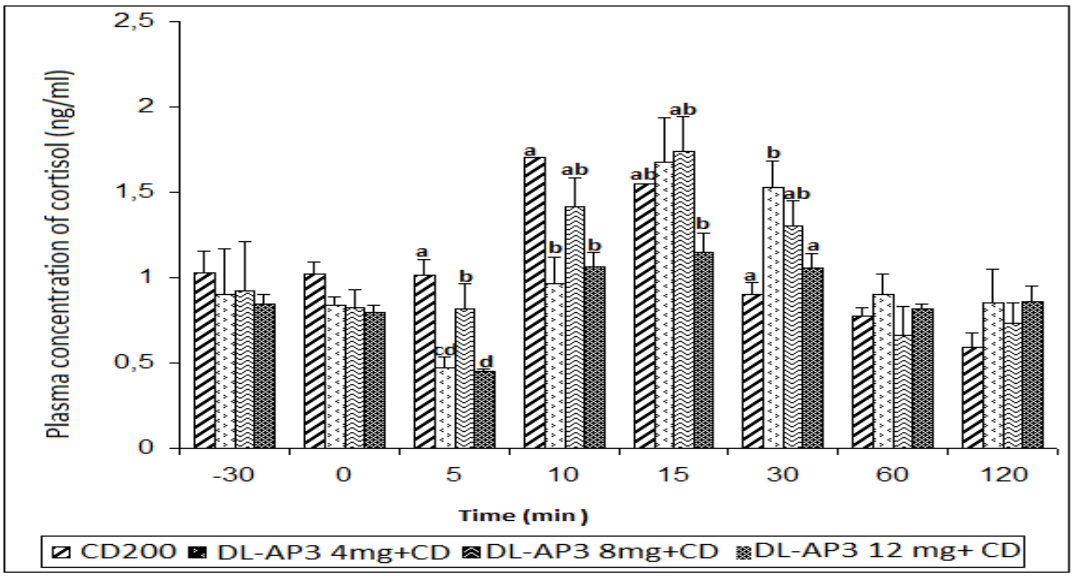

Figure. 1. Comparative analysis of premedication influence with *i.c.v.* DL-AP3 (in doses 4.0, 8.0 and/or12.0 mg/animal) and CD on plasma concentration of cortisol in sheep in comparison with CD200 (x ±SEM, n=6, p≤0,05) , [a, b, c]- different letters indicate statistically significant distinctions when p≤0,05

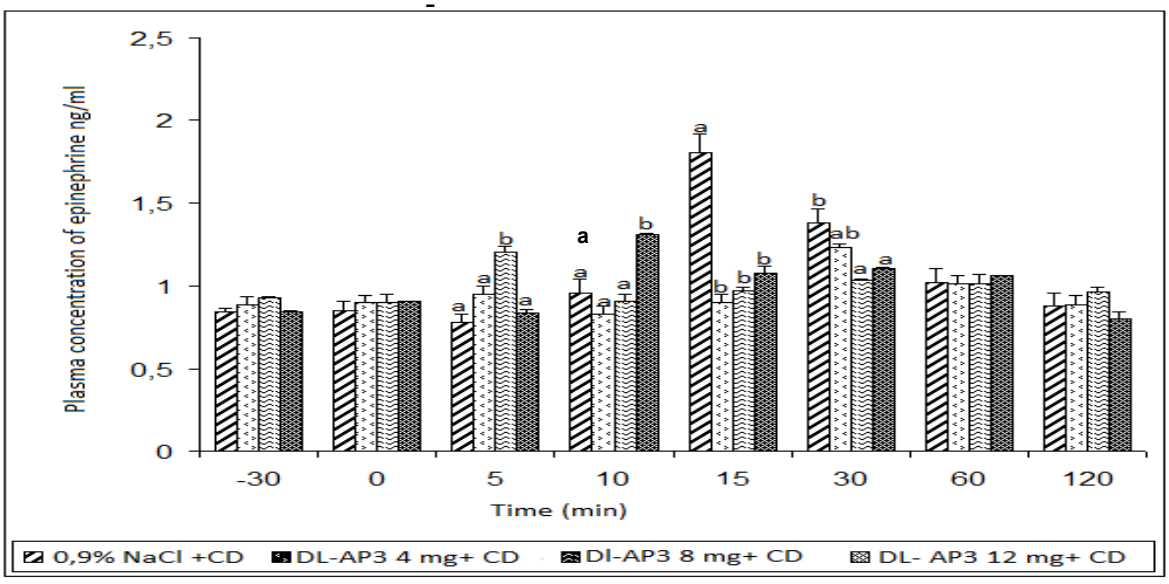

Figure. 2. Comparative analysis of colon distension and different doses of DL-AP3 influence on plasma concentration of E in comparison with RO200 (x±SEM, n=6, p≤0,05), [a, b, c] - different letters indicate statistically significant distinctions when p≤0,05.

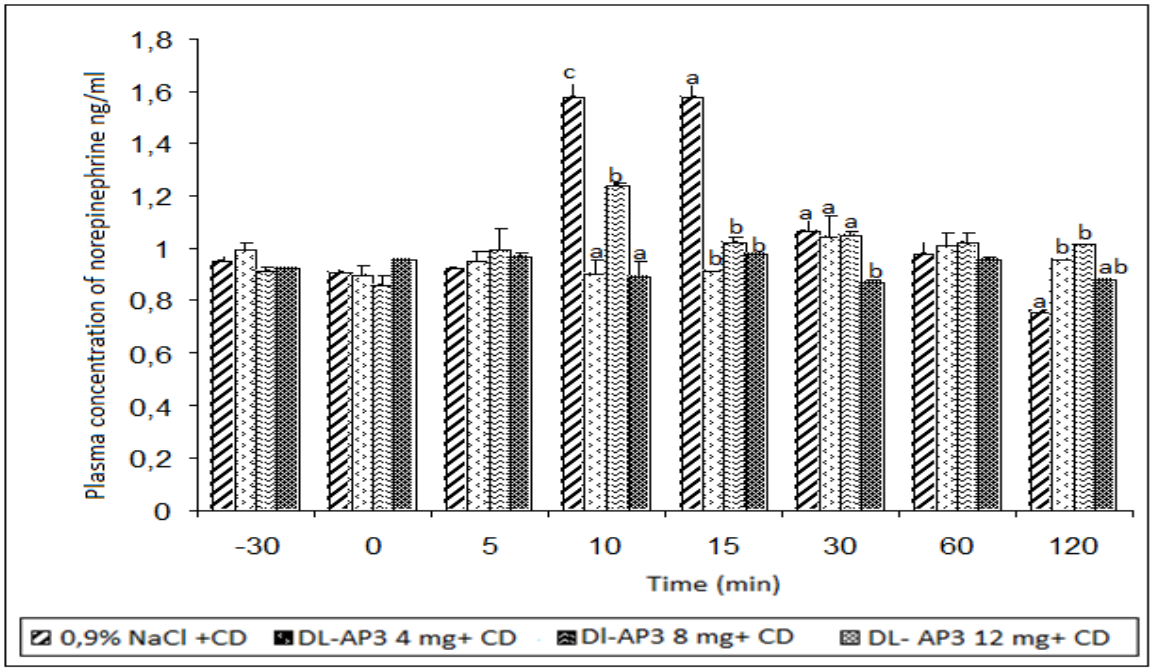

Figure 3: Comparative analysis of colon distension and premedication with different DL-AP3 doses influence on plasma concentration of NE in comparison with CD200 (x ±SEM, n=6, p≤0,05), [a, b, c] - different letters indicate statistically significant distinctions when p≤0,05.

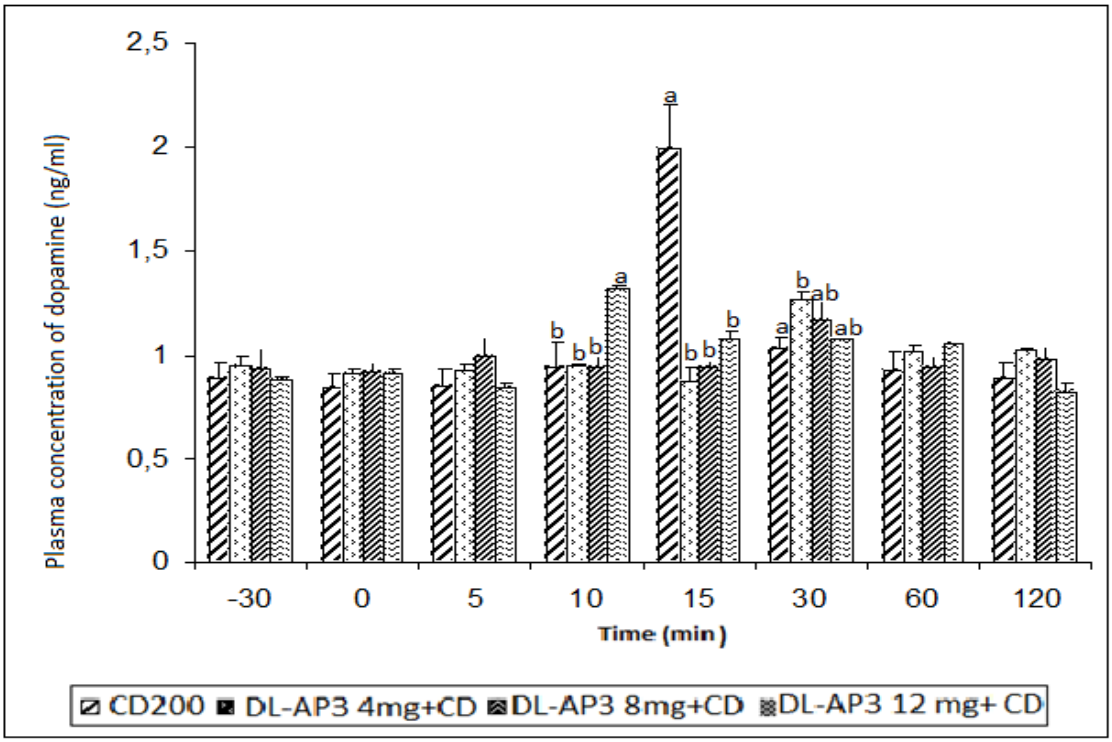

Figure. 4. Colon distension and different DL-AP3 doses influence on plasma concentration of DA (x ±SEM, n=6, p≤0,05), [a, b, c] - different letters indicate statistically significant distinctions when p≤0,05.

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