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THE POSSIBLE ROLE OF SEROTONIN AND ADENOSINE AT DIFFERENT ORIGIN NOXIOUS STIMULI INFORMATION REALIZATION

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Transmission of pain from periphery to the cortex depends on integration and signal processing within spinal cord and higher structures of brain. Several molecular and biochemical mechanisms contribute to the phenomenon of sensitization and persistent pain, wherein every nociceptive factor has its own specific way of action.

The aim of this work is to observe the influence of different noxious agents on serotonin and adenosine level changes in different regions of central nervous system using as a background the modified version of classic formalin test.

We determined the levels of adenosine and serotonin evoked by formalin, capsaicin and Freund's adjuvant injection in the lumbar segment of the spinal cord and hippocampus using HPLC technique.

The role of abovementioned mediators at different levels of nociceptive information transmission is discussed.

pain, modification of formalin test, serotonin, adenosine, high performance liquid chromatography.

Ցավային ազդակների փոխադրումը ծայրամասից դեպի գլխուղեղի կեղև կախված է ողնուղեղում և գլխուղեղի կառուցվածքներում ինտեգրացիայից և ազդակի պրոցեսինգից: Բազմաթիվ մոլեկուլային և կենսաքիմիական մեխանիզմներ հանգեցնում են սենսիտիզացիայի և հարատև ցավի առաջացման, որտեղ յուրաքանչյուր ցավ առաջացնող գործոն ունի իր յուրահատուկ գործելաոճը:

Աշխատանքի նպատակն է դիտարկել տարբեր ցավ առաջացնող ագենտների ազդեցությունը սերոտոնինի և ադենոզինի կոնցենտրացիայի վրա կենտրոնական նյարդային համակարգի տարբեր բաժիններում՝ հիմք ընդունելով դասական ֆորմալինային թեստի մոդիֆիկացված տարբերակը:

Մեր կողմից որոշվել է ֆորմալինի, կապսաիցինի և Ֆրոյնդի ադյուվանտի առաջացրած ցավի արդյունքում ադենոզինի և սերոտոնինի կոնցենտրացիան ողնուղեղի գոտկային բաժնում և հիպոկամպում բարձր արդյունավետության հեղուկային քրոմատոգրաֆիայի եղանակով:

Քննարկված է վերը նշված միջնորդանյութերի դերը ցավային տեղեկատվության փոխանցման տարբեր մակարդակներում:

ցավ, ֆորմալինային թեստի մոդիֆիկացիա, սերոտոնին, ադենոզին, բարձր արդյունավետության հեղուկային քրոմատոգրաֆիա:

Трансмиссия боли с периферии в кору головного мозга зависит от интеграции и процессинга в спинном мозге и высших структурах головного мозга. Различные молекулярные и биохимические механизмы вовлечены в феномен сенситизации и пролонгированной боли, при этом каждый ноцицептивный фактор имеет свой специфический путь влияния.

Целью данного исследования является изучение влияния различных боль-индуцирующих факторов на уровне серотонина и аденозина в различных участках центральной нервной системы с использованием модифицированной модели классического формалинового теста.

Нами было проведено определение уровня серотонина и аденозина в люмбарном сегменте спинного мозга, а также в гиппокампе с применением высокоэффективной жидкостной хроматографии.

Обсуждается роль вышеотмеченных медиаторов на различных уровнях передачи ноцицептивной информации.

боль, модифицированный формалиновый тест, серотонин, аденозин, высокоэффективная жидкостная хроматография.

Introduction

The amazing property of our nervous system is detecting and interpreting a wide range of thermal and mechanical stimuli, environmental and endogenous chemical irritants. When intense, these stimuli generate acute pain, and the setting of persistent injury, both peripheral and central nervous system components of the pain transmission pathway exhibit tremendous plasticity, enhancing pain signals and producing hypersensitivity. When plasticity facilitates protective reflexes, it can be beneficial, but when the changes persist, a chronic pain condition may result. Biochemical, electrophysiological and pharmacological studies are elucidating the molecular mechanisms that underlie detection, coding, and modulation of noxious stimuli that generate pain.

In functioning of nociceptive and antinociceptive systems a special modulating role belongs to different neurotransmitters and meuromediators, particularly serotonin and adenosine.

Serotonin is a mediator in central nervous system, which participates in sensation of pleasure, comfort, regulation of cognitive functions, appetite, sleep/awake processes. The pathways in nervous system which participate in perception of painful signals intersect with those which participate in regulation of emotional status, particularly on serotonin level.

Results of some investigations have shown a spinal analgesic action of 5-HT released from brainstem structures [20, 21]. Since 1969, researchers have investigated stimulation-induced analgesia, which reveals that serotonin (5-HT) excites inhibitory interneurons, resulting in inhibition of dorsal horn neurons [9].

It is shown that the reduction of serotonin concentration leads to attenuation of analgesic effect [3], reduction of pain threshold [10] a higher frequency of pain syndrom development.

Serotonin reuptake inhibitors in certain cases are used to reveal the chronic pain. It is supposed, that the analgesic effect of serotonin can be partly mediated by endogenous opioid system as serotonin promotes the release of beta-endorphin from anterior pituitary cells [22].

Contemporaneously it is proven that exogenous serotonin induces a severe pain in the site of injection. Presumably serotonin along with histamine and prostaglandins plays a role in development of pain impulsation in the locus of injury or inflammation affecting the corresponding receptors in target tissues [19].

The stimulation of large sensory fibers leads to the release of adenosine triphosphate (ATP) from their terminals in posterior horns of spinal cord. The ATP then transforms into adenosine, which affecting the specific A1 receptors blocks the transport of nociceptive information in synapses of thin sensory fibers. However depending on dose adenosine can demonstrate an opposite effect, enhancing nociception. Thus, adenosine is considered as neurotransmitter, which has a modulatory effect on pain formation mechanisms [18].

The aim of this work is to observe the effect of different noxious agents in some regions of nervous system on serotonin and adenosine level using modified model of classic formalin test.

Materials and methods

In this study male albino rats were used weighting 200-250g. The animals were housed in plastic cages by six. The minimal adaptation period was 10 days. The rats were kept in 12 hour light/dark cycle with food and water ad libitum. The experiments were approved by Ethics Committee of Yerevan State Medical University.

In our experiments 7 groups were used: 1. Control group, 2. Single injection of 5% formalin solution, 3. Two subsequent injections of 5% formalin solution with five days

interval, 4. Two subsequent injections of Freund's adjuvant with 5 days interval, 5. Two subsequent injections of capsaicin, 6. Two subsequent injections of 10% and 5% formalin solution respectively with 5 days interval, 7. Two subsequent injections of 5% formalin with 5 days interval, where gabapentin was administrated 10 minutes before the second injection.

Formalin test. In our experiments we used the modification of classic formalin test by adding the second injection of noxious agent with five days interval. Before any injections rats were let in glass chamber for adaptation for 15 minutes. A 5% formalin solution (0.5ml/kg) was subcutaneously injected into the dorsal surface of hind paw. After injection each animal was returned into the glass chamber with a glass in the bottom to observe limb position changes hidden from frontal view. The pain behavior was registered for an hour using a computer program (Lab View, National Instruments) written by us [6], which allows to record animal's per second pain behavior using the scale from 0 to 4, where 0 – there is no pain behavior, 1- the injected paw changes its position but is still in the contact with the floor of chamber, 2- the injected paw is over the chamber floor and there is no contact with any surface, 3- the injected paw is flinched, 4- the injected paw is licked. Pain behaviors are expressed during each period of three minutes intervals during the initial acute phase (0–10 min) or the second, tonic phase (15–60 min). The animals undergo euthanasia immediately after the end of experiment with a Nembutal solution (100mg/kg).

High performance liquid chromatography (HPLC). Samples were taken from rat spinal cord, hippocampus and prefrontal cortex immediately after the decapitation within a minute. All the samples were freezed, then homogenized, centrifuged in 20000 rpm. Sample preparation: a protein precipitation technique was used by adding 3 times more acidified acetonitrile. After the centrifugation with 12000 rpm speed the supernatant was transferred to LC-MS. The mobile phase had the following composition: 0.1% formic acid aqueous solution: acetonitrile 60:40. The flow rate was 0.4 mL/min, injection volume was 10mcL. 268.0-136.0 MRM transition was used for Adenosine MS detection and 177.0-160.0 MRM transition was implied for serotonin MS detection. The calibration curve used covered concentrations between 6.5 ng/ml and 650 ng/ml for quantitation of adenosine, and 10 ng/mL and 10 mcg/mL for quantitation of serotonin.

Staistical analysis. Statistical analysis was performed by the one-way ANOVA (IBM SPSS version 23.0 and MS Excel 2007). A multiple comparison Post Hoc Test (Tukey) was applied to identify groups differing significantly from each other using Bonferroni correction. Data were reported as mean ± SEM.

Results and discussion

As results of our experiments demonstrated, formalin injection into the dorsal surface of the left hind paw led to a two-phase pain response: first phase starts immediately after injection of noxious agent and lasts until 10 min and the second tonic phase, which starts at 15 minutes and lasts until 60 min.

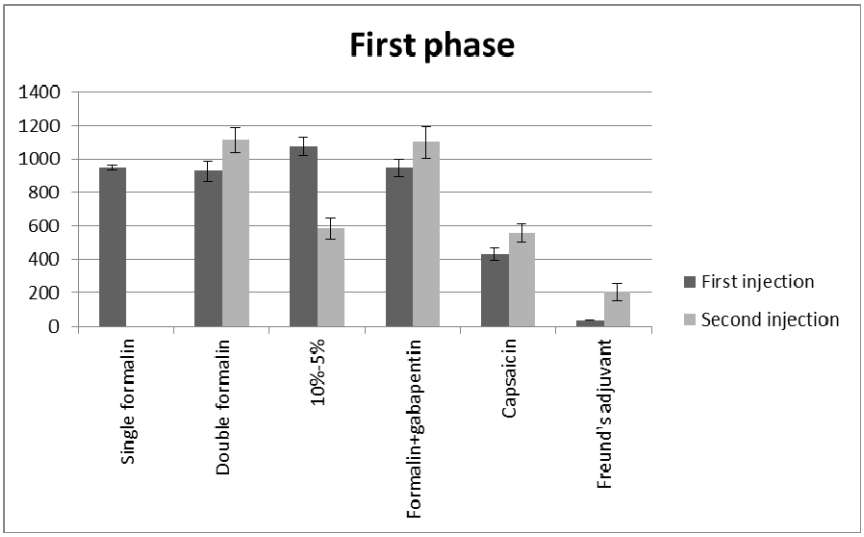

Fig. 1. The value of the pain response (the values shown on vertical axis are obtained by summation of per second pain behavior of experimental animals after injection of noxious agent during the first 15 minutes, where 0 = the injected paw is not favored, 1 = injected paw has little or no weight on it, 2 = the injected paw is elevated and there is no contact with any surface, 3=the injected paw is flinched or shaken, 4 = the injected paw is licked) in the injected paw in the first phase during single and double injections of noxious agent in the modified formalin test.

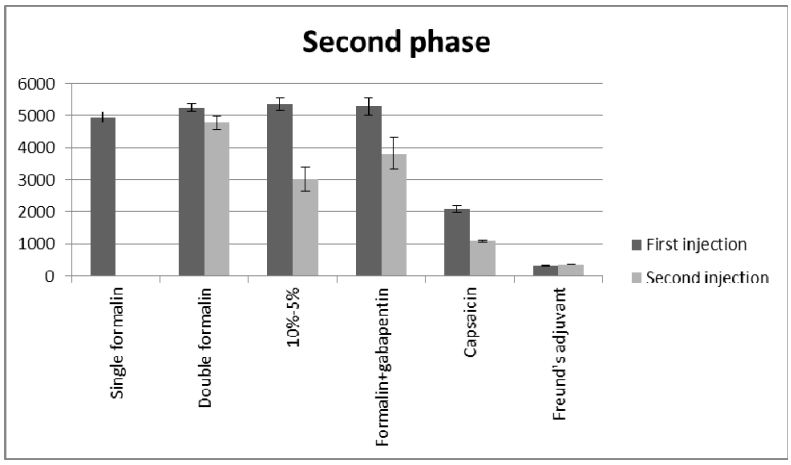

Fig. 2. The value of pain response after formalin injection during the last 45 minutes (the pain intensity is calculated as in Fig. 1) in the injected paw in the second phase during single and double injections in the modified formalin test.

As it can be seen from the results, all the noxious agents (formalin, capsaicin, Freund's adjuvant) demonstrated similar patterns in both phases. In the first phase, in double formalin group, the value of the second injection was higher. The formalin+gabapentin group did not demonstrate any changes in first phase, which is in agreement with literature data [1, 16], indicating that gabapentin does not affect the acute phase in formalin test. In the group with subsequent injection of 10% and 5% formalin the first injection value is higher as it could be expected, but in the second injection the pain value significantly lowers. Both, capsaicin and Freund's adjuvant, demonstrate a similar pattern with formalin, showing lower values.

Concerning to the second phase, in almost all groups the second injection value was lower, than the first one. The lowest value compared with the first injection reveals the group with subsequent injections of 10% and 5% formalin. Gabapentin significantly lowered the second phase of the formalin test.

HPLC results

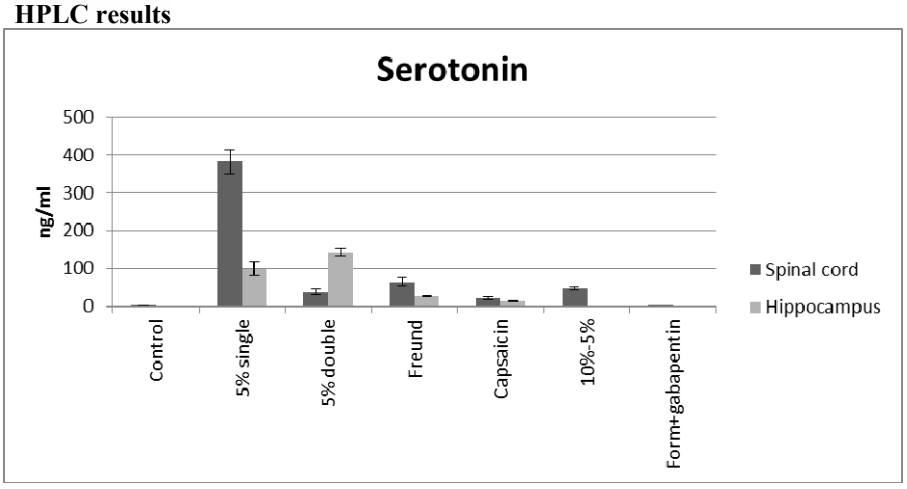

Fig. 3. The quantitative definition of serotonin in rat spinal cord and hippocampus measured by HPLC

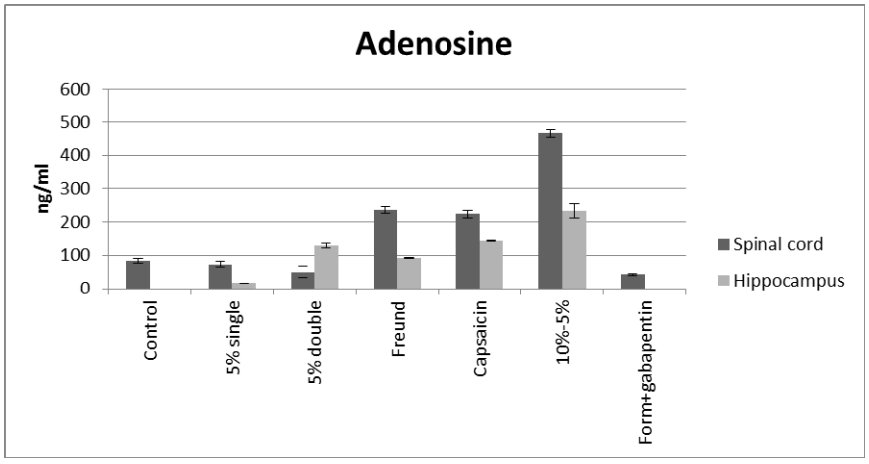

Fig. 4. The quantitative definition of adenosine in rat spinal cord and hippocampus measured by HPLC

As figure 3 show, the highest level of serotonin can be observed in the group with single injection of 5% formalin. The injection of formalin increases serotonin level both in spinal cord and hippocampus [17].

The slight changes of serotonin level were observed in all the other groups. Some literature data indicate, that the injection of Freund's adjuvant significantly increases the serotonin level both in spinal cord and in hippocampus [4]. The minor changes can be explained presumably by insufficient time for inflammation to develop. In the group with subsequent injection of 10% and 5% formalin the low concentration of serotonin can be explained most likely by the increased pain threshold, which is likewise observed in formalin test in both first and second phases.

Interestingly in formalin+gabapentin group the serotonin level was about zero, despite some works indicate, that gabapentin increases blood serotonin [12].

Gabapentin decreased the serotonin level both in spinal cord and in hippocampus. However, several observations are consistent with the idea that gabapentin modulates Ca^{2+} channels, particularly if channels are modulated in a subtle manner. It is possible, that inhibition of monoamine neurotransmitter release [2,13,15] is caused by an interaction of gabapentin with Ca^{2+} channels. However, there are there are some results, indicating that gabapentin increases the concentration of serotonin in whole blood [12]. These authors speculate, that increased serotonin might be due to changes in serotonin metabolism or uptake in platelets.

The inhibitory action of serotonin on structures of the dorsal horn may be mediated by activation of opioid-releasing interneurons. In animal models the opioid antagonists attenuate the analgesic effect of intraspinal serotonin; similarly, serotonin antagonists interfere with analgesic effects of morphine infused in or near the spinal cord [8].

Serotonin is released in spinal cord by descending systems that modulate somatosensory transmission and can potently depress primary afferent-evoked synaptic responses in dorsal horn neurons.

Additionally, serotonin receptor antagonists, given to rats intrathecally, inhibited experimental pain response [5], suggesting, that excitatory serotoninergic descending pathways facilitate the expression of pain. It is likely, that serotonin inhibits, as well as promotes pain perception by different physiological mechanisms [7].

As it can be seen from Fig. 4, the highest level of adenosine is observed in the group with subsequent injection of 10% and 5% formalin both in spinal cord and hippocampus. Interestingly, the concentration of adenosine lowered while administrating gabapentin.

Adenosine regulates pain transmission in the spinal cord and in the periphery, and a number of agents can alter the extracellular availability of adenosine and subsequently modulate pain transmission, particularly by activation of adenosine A1 receptors.

Moreover, there is a significant increase of adenosine level in Freund's adjuvant and capsaicin groups in spinal cord and hippocampus. The use of capsaicin (which activates receptors selectively expressed on C-fibre afferent neurons and produces neurotoxic actions in certain paradigms) allows for an interpretation of C-fibre involvement in such processes. In the spinal cord, adenosine availability/release is enhanced by depolarization (K^+, capsaicin, substance P, N-methyl-D-aspartate (NMDA)), by inhibition of metabolism or uptake (inhibitors of adenosine kinase (AK), adenosine deaminase (AD), equilibrative transporters), and by receptor-operated mechanisms (opioids, 5-hydroxytryptamine (5-HT), noradrenaline (NA)). Some of these agents release adenosine via an equilibrative transporter, indicating production of adenosine inside the cell (K^+, morphine), while others release nucleotide which is converted extracellularly to adenosine by ecto-5'-nucleotidase (capsaicin, 5-HT). Release can be capsaicin-sensitive, Ca^{2+}-dependent and involve G-proteins, and this suggests that within C-fibres, Ca^{2+}-dependent intracellular processes regulate production and release of adenosine. In the periphery, adenosine is released from both neuronal and non-neuronal sources. ATP is released both spinally and peripherally following inflammation or injury, and may be converted to adenosine by ecto-5'-nucleotidase contributing an additional source of adenosine. Release of adenosine from both spinal and peripheral compartments has inhibitory effects on pain transmission [14].

Conclusion

The pain information in the CNS is controlled by ascending and descending regulatory systems, in which endogenous substances play a modulatory role.

Serotonin acts substantially on spinal cord level, and affects descending pain pathway. It is important to mention, that classic formalin test, which proposes a single injection of formalin, significantly increases serotonin level in spinal cord. So it can be considered as "early" stimulation-produced mediator.

Possibly, the stimulus intensity is also related with serotonin release. Interestingly it is in agreement with our results of formalin test. It represents the second phase. In formalin test all the second injections are lower compared with the first injections. Concerning to adenosine, it is possibly a "late" mediator. Adenosine represents the first phase of formalin test respectively with first and second injections.

Presumably, regulation of descending inhibitory modulation proposes, that or higher brain areas directly communicate with descending nociceptive fibers, either this communication is polysynaptic, acting through intermediate relays.

So our results let us to conclude, that the route from higher brain areas is not direct and depends on variety of factors, i.e. stimulus intensity, type of injury, number of injections, thus, the intermediate levels of descending pain pathway can undergo changes, affecting the level of mediators at spinal cord level.

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THE CHANGE OF POLYAMINES AND NITRIC OXIDE QUANTITIES IN HUMAN BLOOD SERUM OF PROSTATE AND BLADDER CANCER

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Currently are shown rapid growth of polyamines and nitric oxide (NO) quantities in blood serum during malignant tumors in different organs. Increased NO generation in cancer cells may contribute to tumor angiogenesis and metastatic ability by up-regulating vascular endothelial growth factor. The goal of workwas to researchin human blood serum the changes of polyamines and NO quantities in different stages of prostate and bladder cancer. Polyamines and NO quantities were determined in blood serum of 11 healthy individuals (34-63 years old) and patients with prostate (28) and bladder (20) cancers (48 patient,I-III stages, 44-73 years old) who were hospitalized in the National Centre of Oncology RA aft. V.A. Fanarjyan. Total polyamines quantity compared with standard was increased by42.5%, 54.5% and 76.8%, respectively in I, II and IIIstages of prostate cancer, and 59.5%, 64.8% and 87.9%, respectively in I, II and IIIstages of bladder cancer.The quantity of nitrite anions was increased by 125% in prostate and bladder cancers patients blood serum.The increase of NO and polyamines concentrations in blood serum in earlier stages and the increase in parallel to cancer development confirm, that this metabolic pathway of L-arginine has a significant role in promoting tumor growth and development. We suggest that downstream of polyamines and NO quantities might have antitumor effect on cancer development.

cancer, polyamine, NO, arginase, tumorigenesis, antitumor potential