THE BRAIN TISSUE DEHYDRATION AS A MECHANISM OF ANALGESIC EFFECT OF HYPERTONIC PHYSIOLOGICAL SOLUTION IN RATS

Sinerik Ayrapetyan[1], Gohar Musheghyan[2], Anush Deghoyan[3]*

1. Prof. UNESCO Chair-Life Sciences International Postgraduate Educational Center, 31 Acharyan St. 0040 Yerevan, Armenia.
2. PhD. UNESCO Chair-Life Sciences International Postgraduate Educational Center, 31 Acharyan St. 0040 Yerevan, Armenia.
3. MS. UNESCO Chair-Life Sciences International Postgraduate Educational Center, 31 Acharyan St. 0040 Yerevan, Armenia.

Abstract

Previously were shown that neuronal shrinkage in hypertonic solution causes the decrease of the number of functional active protein molecules in membrane having channel forming, receptor and enzyme properties. On the basis of this data the correlation between rats' brain tissue hydration and the number of ouabain receptors in membrane and pain threshold to the "hot plate" were studied.

For the estimation of brain tissue hydration the differences between wet and dry weights of tissue were measured. The number of functionally active ouabain receptors was determined by counting the number of binding labeled molecules of ouabain-3H in the brain tissue. Pain threshold was determined by means of hot plate test.

Hypertonic solution caused the decrease of brain tissue hydration and the number of ouabain receptors and the increase of pain threshold. Time-dependent increase of brain tissue hydration and decrease of pain threshold were observed.

On the basis of the obtained data we concluded that the pain-relieving effect of hypertonic solution is due to the decrease of the number of functionally active protein molecules in cell membrane determining its excitability. ***(J Int Dent Med Res 2010; 3: (2), pp. 93-99)***

Keywords: Rat, cell hydration, pain threshold.

Received date: 05 February 2010 **Accept date: 07 May 2010**

Introduction

The hypertonic saline (HTS) is an osmotic agent which can help patients in the acute phase of severe traumatic brain injury (TBI) and it can diminish the effects of the secondary brain injury in patients with TBI. However, the molecular and cellular mechanisms underlying the HTS-induced therapeutic effect on TBI are still unknown. The elucidation of these mechanisms could also have considerable clinical impact, especially since osmolarity disturbances are described in various diseases such as diabetes [1], alcoholism [2] and aquadynia [3].

In our previous works performed on snail single neurons we showed the close correlation between cell volume and membrane excitability, chemosensitivity and enzymatic activity: cell swelling leads to the increase in the number of functionally active protein molecules in the membrane, which have channel forming, receptor and enzymatic properties, while the cell shrinkage decreases its number [4-6]. On the basis of these data a hypothesis was suggested, according to which the cell overhydration-induced generation of neuromembrane abnormal excitation could serve as a nociceptive signal for the central nervous system [7- 9]. According to this hypothesis the pain-relieving effect of hypertonic solution is explained by the decrease of brain sensitivity (namely cortex neurons) to the input signals as a result of cell shrinkage-induced depression of neuromembrane functional activity. For testing this hypothesis the dependence of

Corresponding author:
Prof. Sinerik Ayrapetyan,
UNESCO Chair-Life Sciences International Postgraduate Educational Center
31 Acharyan str.
Yerevan, 375040, Armenia

E-mail: info@biophys.am

pain threshold to the hot plate on brain tissue hydration and the number of ouabain receptors (markers for membrane active surface) in rats interperitoneally injected to physiological solution (PS) with different tonicities as well as in rats preliminary provided with distilled water (DW) (instead of regular drinking water) *ad libitum* during 3 and 5 days, were studied.

The obtained data in the present work allowed us to suggest that the analgesic effect of hypertonic solution on rats is determined by the decrease in the number of functionally active protein molecules in brain cells caused by cell shrinkage.

The close correlation between cell hydration and the number of ouabain receptors (Na,K-ATPase molecules) in cell membrane was shown in our previous works[6,11]. The cell swelling in hypertonic and shrinkage in hypertonic mediums caused the increase and the decrease of the number of oubain receptors in the cell membrane, correspondingly. The study of the dose-dependent effects of ouabain on the Na^+/K^+ pump activity showed that high (more than 10^{-7} M) concentrations of ouabain inhibited the pump activity [6], while at lower concentrations it had no effect on pump function [7]. Therefore, the number of ^{3}H-ouabain receptors in the membrane at its concentration of less than 10^{-7} M was suggested as a new and extrasensitive membrane marker for determining the size of the membrane active surface which correlated with the cell volume [6, 11]. In these experiments the correlation between rats' brain tissue hydration and the number of ouabain receptors in membrane and pain threshold to the "hot plate" were studied for revealing the mechanism(s) through which the pain-relieving effect of hypertonic solution is realized.

Materials and Methods

All the procedures performed on the animals were carried following the protocols approved by the Animal Care and Use Committee of LSIEPC.

Animals

All the experiments were performed on naive male unstrained albino rats (Wt 150-200 g). The animals were housed under optimum conditions of 12 hour-light/dark cycle and 22 ± 2 ^{0}C temperature with food and water access *ad libitum*. The brain tissue hydration was provoked by providing the animals with DW *ad libitum* during 3 and 5 days or by single application of interperitioneal injections of 3 ml DW, isotonic and hypertonic (2 M Mannitol containing) PS.

Chemicals

Thyrode solution (in mM: NaCl (137); KCl (5,4); $CaCl_2$ (1,8); $MgCl_2$ (1,05); $C_6H_{12}O_6$ (5); $NaHCO_3$ (11,9); NaH_2PO_4 (0,42); pH 7.4) served as a normal PS for the experiments. The tissue dehydration was provoked by concentrated osmoduretics mannitol [C_6H_8 $(OH)_6$, mol. Wt.=182,18], 2 M dissolved in 3 ml normal saline. In control experiments the animals were treated by 3 ml isotonic saline injection. The intraperitoneally injections of mannitol (n=56) and saline (n=11) were performed 30 min. before the experiments.

Tissue preparation

First of all the possible effects of emotional stress and pain sensation (arising at the ordinary technique of intraperitoneally PS injections, animal decapitation during the forcible immobilization of the awaked animal) on the baseline level of water in brain tissue were determined in non-anesthetized and anesthetized animals. Water content and the number of ouabain receptors in different organs and in different brain zones were determined in intact and testing (saline injected) rats. The decapitation of rats was performed after their anesthetization by Diphenyl ether. In order to stop the brain metabolism, the removed brain was dipped into liquid nitrogen for 15 sec. For the estimation of the saline injection-induced pain sensation on brain tissue hydration, the non-anesthetized animals (n=5) were decapitated after their sharp immobilization by dipping the head into liquid nitrogen (3-4 sec). After such procedures the full absence of somatic reflexes on extra stimuli was recorded. The testing tissue slices (thickness ~0,5mm) of heart (n=10), kidney (n=10), liver (n=10), spleen (n=10), brain cortex (n=10), stem (n=10) and cerebellum (n=10) were taken from each rat.

Definition of tissue water content

After measuring the wet weight (w.w.) of brain tissue samples, the latter were dried in thermostat (T-121, Russian production) at 105^{0}C during 24 hours for determination of dry weight

(d.w.). The quantity of water in 1 g of d.w. was counted by the following equation: (w.w. – d.w.) / d.w. (10) and expressed as water content in grams per gram of dry weight.

Counting the number of ouabain receptors in cell membrane of different brain tissues

Brain tissue slices with 1mm thickness were prepared by microtome MT-23 (Russian production). Brain tissue samples (ten from each tissue of one rat) were incubated in testing saline containing 10^{-8} M [^{3}H]ouabain (12 Ci/mM activity), and then washed threefold for 10 min with the same concentration of testing saline containing unlabeled ouabain molecules to remove the tritium-labeled ouabain absorbed into the extracellular space and not connected with the receptors [6]. The tissue pieces were placed in special vials and homogenized with HNO_3. Finally, 5 ml of Bray's scintillation fluid was added, and the mixture was counted in a Wallac-1450 liquid scintillation counter (Perkin Elmer, Finland).

Determination of pain threshold

The hot plate test was conducted by a specific setup constructed in our laboratory and approved by the Ethic Committee of UNESCO Chair in Life Sciences. The setup consists of the org-glass chamber with the brass bottom. The temperature of the bottom (51^0C) was controllable and a thermometer (accuracy of measurement $\pm 0{,}01^o$C) was placed on it. In order to keep the temperature constant, the brass bottom was completely covered by the Plexiglas box.

A rat was placed in this chamber and pain latency was recorded. Latency (in sec) is defined visually as the time elapsed until one of the following responses: licking the feet, jumping or rapidly stamping the feet. For preventing the tissue damage the cut-off time was chosen 50 sec.

Statistical analysis

The Microsoft Excel and Sigma-Plot (Version 8.02A) were used for the data analysis.
The mean value and standard error of hydration index of different tissues and pain threshold were calculated and the statistical probability was determined by Student's t-test with the help of the computer program Sigma-Plot (Version 8.02A). The statistical probability was expressed on figures with the help of asterisks (*).

Results

The level of tissue hydration of different organs of rats, including brain, can be changed by the intraperitioneally injection of DW or PS of different tonicities, or by giving the rats DW instead of regular drinking water during 3 and 5 days.

To find out the emotional stress effect of the saline injection procedures on rat brain tissue hydration, the effect of 3 ml of isotonic PS injection on brain tissue hydration in anesthetized and non-anesthetized animals was studied. As can be seen in Figure 1, in anesthetized group of animals (n=10) the saline injection had no significant effect on brain tissue hydration (Figure 1A), while in non-anesthetized animals it led to the cortex hydration by 10%±1.32 and to the stem and cerebellum tissue dehydration by 15,8%±1.77 and 5,5%±2.13, correspondingly, as compared with the control (non-injected animals) group (Figure 1B). The sensitivity of brain tissue hydration to the injection of isotonic solution in non-anesthetized animals could be explained by the injection-induced pain sensation of animals. The differences between the responses of various parts of the brain to the injection-induced stress could be explained by their different functional role in stress responses of organism, which could be the subject for a special investigation.

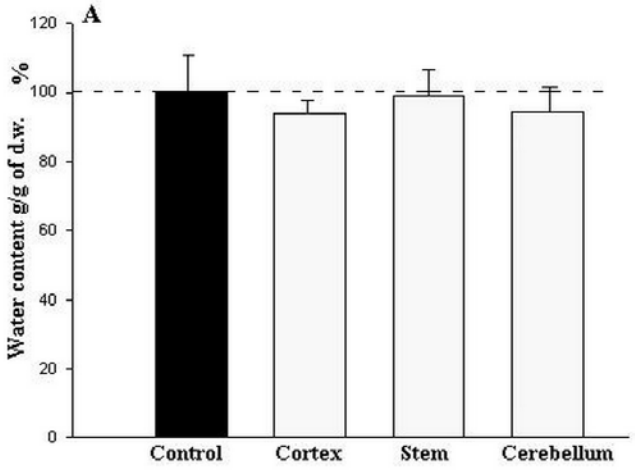

Figure 1a.

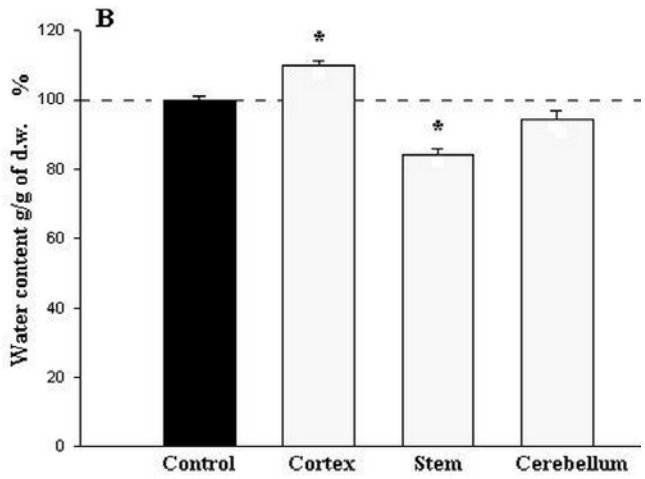

Figure 1b.

Figure 1ab.The effect of 3 ml isotonic PS interperitoneal injection on tissue hydration of different brain zones in anesthetized (a) and non-anesthetized (b) animals, expressed in %, compared to the data of intact animals. *P<0.05.

For studying of the correlation between tissue hydration and pain threshold in rats, the experimental animals were divided into six groups, per 10 animals in each group: control-1-intact; Control-2 -animals intraperitoneally injected of 3 ml isotonic PS; animals intraperitoneally injected of hypertonic solution (Mannitol); and DW, as well as the intact animals whose were given DW instead of regular drinking water for 3 and 5 days. In all control and experimental groups the pain threshold to the hot plate was determined before the dissection.

From the data presented in Table 1 we can see that intraperitoneally injection of 3 ml DW led to brain tissue hydration that was accompanied by the increase in the number of ouabain receptors in it. Such DW injection led to the increase of brain cortex, stem, and cerebellum hydration by 42%±2.37, 34.6%±2.75, 23%±3.075 and the number of ouabain receptors- by 45%±2.45, 38%±2.76, 23%±1.13, correspondingly, compared with the data of Control-2 (isotonic PS injected) animals. The intraperitoneal injection of hypertonic solution (Mannitol) led to the brain tissue dehydration: cortex- 26,5%±0.69, stem- 12,3%±0.69 and cerebellum- 17,5%±0.73 and the decrease in the number of ouabain receptors: cortex- 27%±1, stem- 10.2%±0.55, cerebellum- 23%±0.9, correspondingly. These data clearly show that there are close correlation between brain tissue hydration and the number of functionally active ouabain receptors in the cell membrane that could serve as a marker for other membrane proteins, like as ionic channels and receptors. The fact that the brain cortex hydration is more sensitive to the osmotic stress than the stem and cerebellum tissues hydration could indicate on the crucial role of the cortex in stress sensation of the organism rather than other brain zones.

To find out the correlation between brain cortex hydration and pain threshold the measurements of the pain threshold of each animal before and after 30 min of test solution injection were performed. Because of a big variation of the initial value of pain thresholds in different animals, the factor induced changes of pain threshold compared with the initial one were expressed in %.

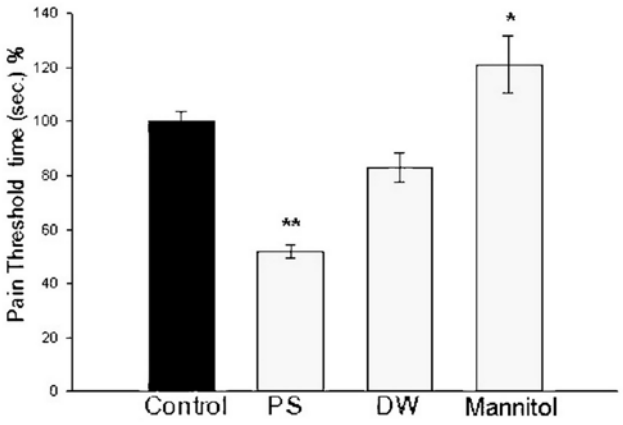

Figure 2. The pain threshold of rats to the "hot plate" before (Control) and after interperitioneal injection with isotonic (PS), hypertonic (2 M Mannitol) physiological solutions and distilled water (DW), expressed in % compared to the initial pain threshold of the same animal (n=10). *P<0.05; **P<0.01.

As shown in Figure 2, the pain thresholds (latent period) to the hot plate of rats injected to isotonic PS, and DW were decreased by 48,2%±2.34 and 17%±5.41, correspondently, while in hypertonic solution (Mannitol) injected animals it was increased by 21%±5.41 (n=10). The fact that in rats injected to isotonic PS, the pain threshold was decreased can be explained by the emotional stress effect on animals to the injection procedures, which was accompanied by the increase of brain cortex hydration (Fig 1). The DW injection had double effect on pain threshold;

from one hand there was a dilution of extracellular Na ion concentration that caused the decrease of membrane excitability, from the other hand the dilution of extracellular medium caused the cell swelling. To avoid the injection-induced side effects, in the next series of experiments the correlation between brain tissue hydration and pain threshold was studied on two groups of animals which were provided with DW (instead of normal drinking water) *ad libitum* during 3 and 5 days. As can be seen on Table 2, in animals provided with DW there are time-dependent increase of brain tissue hydration and the increase on the number of ouabain receptors in it, compared to the brain tissue hydration of animals provided with normal drinking water. It is worth to note that as in previous series of experiments again the cortex tissue hydration was more sensitive to DW than brain stem and cerebellum tissues hydration. The comparative study of the pain threshold of rats before and after 3 and 5 days of providing the DW as drinking water can be seen on data presented in Figure 3, where the time-dependent decrease of pain threshold was observed. Such negative close correlation between brain tissue hydration (number of ouabain receptors) and pain threshold could serve as a strong evidence for the working hypothesis of the present work that the pain-relieving effect of hypertonic saline can be explained by dehydration–induced depression of neuromembrane excitability in brain cortex tissue.

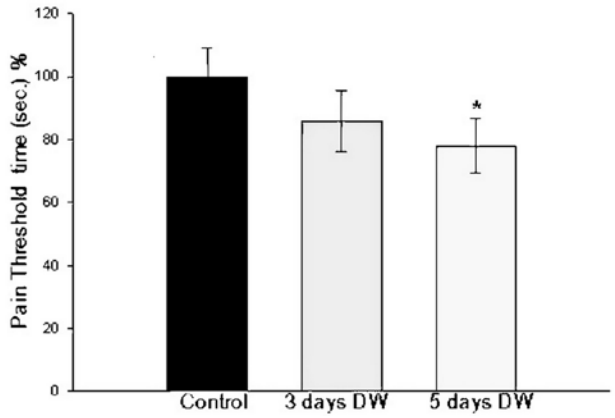

Figure 3. The pain threshold of rats to "hot plate" before (Control) and after 3 and 5 days of providing with distilled water (DW) instead of drinking water, expressed in % compared to the initial pain threshold of the same animal. *P<0.05.

Discussion

Hyperosmolar therapy is one of treatment interventions in the care of patients with severe head injury resulting in cerebral edema and intracranial hypertension. However, the cellular and molecular mechanisms underlying the therapeutic effect of hypertonic solution are not clear yet. Earlier studies have shown that the metabolic controlling cell hydration is a dynamic parameter determining the cell functional activity, like membrane excitability, chemo sensitivity, enzyme activity[12, 13]. Even during the generation of a single action potential the cell swells and shrinks: at the ascending phase of action potential (depolarization) the squid's giant axon and crab's nerve fibers swell [14]. The cell swelling also takes place as a result of electrical and chemical substances-induced increase of membrane excitability [6] or cell poison [15-17]. As the close correlation between cell hydration and membrane excitability is well documented on single isolated neurons, the cell over hydration was suggested as a messenger for nociceptive signals generation[7-9].

The cardiac glycoside–ouabain, which is traditionally considered as a specific inhibitor for Na^+/K^+ pump, has high affinity receptors in neuronal membrane (at concentration of less than 10^{-7} M), the function of which is not connected with the function of Na^+/K^+ pump [6,13]. The number of these receptors as the other membrane proteins is in functionally active and inactive (reserve) states, depending on cell hydration[6]. Therefore the number of binding labeled molecules of ^{3}H-ouabain to cell membrane at its concentration of less than 10^{-7} M was suggested as a marker for estimation of cell membrane active surface (cell hydration)[11].

The obtained data in the present work clearly demonstrate that there is a close correlation between tissue hydration and number of ouabain receptors (marker for membrane proteins) in the membrane: the increase of tissue hydration leads to the increase in the number of ouabain receptors, while the hypertonic PS application has opposite effect on it (Tables 1 and 2).

The fact that the increase of animal brain tissue hydration and number of ouabain receptors (Table 1 and 2) decrease the pain thresholds to hot plate, while its dehydration and decrease of number of ouabain receptors hava opposite effect on pain threshold (Figure 2 and 3)

confirms our early hypothesis according which the nerve cell over hydration serves as a messenger for pain signal generation ([7-9]).

The data on the increase of brain cortex hydration and number of ouabain receptors, in response to the isotonic PS-injection in non-anesthetized animals (Figure 1B), which was absent in anesthetized animals (Figure 1A) can be explained by direct influence of the injection-induced pain sensation on cortex tissue hydration. The fact that in non-anesthetized animals, after isotonic PS injection we observed also the decrease of pain threshold (Figure 1) indicates on the existence of close correlation between cell hydration in cortex and pain thresholds in rats.

A comparatively higher sensitivity of the brain cortex hydration to osmotic stress induced by injection of hypertonic solution and DW, as well as to the animals, to which DW was provided as a drinking water (Tables 1 and 2), indicates the crucial role of cortex in stress reaction of organisms.

Conclusions

Thus, the obtained data in the present work and our early data obtained on single snail neurons and squid axons [13], allow us to conclude that the analgesic effect of hypertonic saline on brain trauma (over-hydrated cells) is due to dehydration-induced cell shrinkage of brain cortex cells, causing the depression of membrane excitability as a result of the decrease in the number of functional active proteins in the membrane.

Acknowledgements

The authors thank to Ms. Armenuhi Hekimyan for technical assistance.

Brain Zones	*Control 1 (intact animals)*	*Control 2 (isotonic PS injection)*	△%	P value	*DW injection*	△%	P value	*Hypertonic PS (Mannitol) injection*	△%	P value
Hydration [water cont. g/g d.w.] (mg.)										
Cortex	2.82±0.05	3.1±0.0374	↑ 10	P =0,0000078	4.4±0.074	↑ 42	P = 0,4	2.28±0.022	↓ 26.5	P = 0,00039
Stem	3.09±0.075	2.6±0.055	↓ 15.8	P = 0,000025	3.5±0.072	↑ 34.6	P = 0,3	2.28±0.018	↓ 12.3	P = 0,0006
Cerebellum	2.75±0.041	2.6±0.06	↓ 5.5	P = 0,0001	3.2±0.08	↑ 23	P = 0,8	2.145±0.019	↓ 17.5	P = 0,053
Number of ouabain receptors (x10^7 molecule g/ d.w.)										
Cortex	19± 0.18	20.9±0.13	↑ 10	P =0,0000067	30.4±0.027	↑ 45	P = 0,011	15.2±0.8	↓ 27	P = 0,00025
Stem	17± 0.22	14.3±0.16	↓ 15.8	P = 0,000033	19.8±0.021	↑ 38	P = 0,013	12.84±0.5	↓ 10.2	P = 0,0001
Cerebellum	22± 0.2	20.87±0.3	↓ 5.1	P = 0,0005	25.69±0.04	↑ 23	P = 0,1	16.05±0.9	↓ 23	P = 0,034

Table 1. The tissue hydration and number of ouabain receptors of different brain zones in intact (Control 1), isotonic PS injected (Control 2), DW and hypertonic PS (Mannitol injected) animals. The experimental results were compared with control 2 group of animal. Each group of animal was consist of 10 animal. The difference between control 2 and experimental groups was statistically significant (***P<0.001).

Brain Zones	Control	3 days of DW	△%	P value	5 days of DW	△%	P value
Hydration [water cont. g/g d.w.] (mg.)							
Cortex	2.78±0.05	3.03 ± 0.04	↑ 8	P = 0,0005	3.28 ± 0.05	↑ 18	P = 0,00002
Stem	2.57±0.075	2.61 ±0.05	↑1.5	P = 0,09	2.8 ± 0.04	↑ 9	P = 0,0073
Cerebellum	2.19±0.041	2.31 ± 0.05	↑5.5	P = 0,1	2.43± 0.07	↑ 11	P = 0,04
Number of ouabain receptors (x10^7 molecule g/ d.w.)							
Cortex	18± 0.18	19.4 ± 0.1	↑7.8	P = 0,0007	21.35 ± 0.1	↑18.61	P = 0,000012
Stem	13± 0.22	13.16 ±0.15	↑1.2	P = 0,08	14.18 ± 0.12	↑ 9	P = 0,0062
Cerebellum	20± 0.2	21.10± 0.25	↑5.5	P = 0,13	22.20± 0.35	↑ 11	P = 0,032

Table 2. The tissue hydration and number of ouabain receptors of different brain zones in intact animals (Control), provided with normal drinking water and animals provided with DW during 3 and 5 days. The experimental results were compared with control group. Each group of animal was consist of 10 animal. The difference between control 2 and experimental groups was statistically significant (***P<0.001).

References

1. Puliyel JM, Bhambhani V. Ketoacid levels may alter osmotonicity in diabetic ketoacidosis and precipitate cerebral edema. Archives of Disease in Childhood 2003;88:366
2. Vamvakas S, Brüning T, Thomasson B. Renal cell cancer correlated with occupational exposure to trichloroethylene. J Cancer Res Clin Oncol. 1998; 124: 374–82.
3. Misery L, Meyronet D, Pichon M, Brutin JL, Pestre P, Cambazard F. Aquadynia: a role for VIP? Ann Dermatol Venereol 2003; 130: 195–8.
4. Ayrapetyan SN, Rychkov GY, Suleymanyan MA. Effects of Water Flow on Transmembrane Ionic Currents in Neurons of Helix Pomatia and in Squid Giant Axon. Comp Biochem Physiol 1988; 89(2): 179-186.
5. Ayrapetyan SN, Arvanov VL, On the mechanism of the electrogenic sodium pump dependence of membrane chemosensitivity. Comp Biochem Physiol 1979; 64(A): 601-604.
6. Ayrapetyan SN, Suleymanyan MA, Sagian AA, Dadalyan SS. Autoregulation of Electrogenic Sodium Pump. Cell Mol Neurobiol 1984; 4: 367- 384.
7. Ayrapetyan SN. Cellular Mechanism of pain. In: Kepplinger B, Ray AD, Schmid H, eds. Pain- Clinical Aspects and Therapeutical Issues, Edition Selva Verlag, Linz, Austria, 1995; 311-327.
8. Ayrapetyan SN. Theoretical aspects of magnitotherapy of pain. Abstr. of the 7th Int. Symposium "The Pain Clinic", Istanbul, Turkey, 1996: 171-2.
9. Ayrapetyan SN. The application of the theory of metabolic regulation to pain. In: Ayrapetyan SN, Apkarian AV, eds. Pain Mechanisms and Management. IOS press, Netherlands, 1998; 3 - 14.
10. Adrian RH. The effect of internal and external potassium concentration on the membrane potential of frog muscle. J Physiol 1956; 133: 631–658.
11. Danielian A.A., Ayrapetyan S.N. Changes of Hydration of Rats' Tissues after in Vivo Exposure to 0.2 Tesla Steady Magnetic Field. Bioelectromagnetics, 1999; 20(2): 123-128.
12. Parton G.R. and Simons K. The multiple faces of caveolae. Nature Reviews, 2007; 8: 185-194.
13. Ayrapetyan SN. On The Physiological Significant of Pump Induced Cell Volume Changes. Adv Physiolo Sci 1980; 23: 67-82.
14. Iwasa K, Tasaki I, Gibbons RC. Mechanical changes in crab nerve fibers during action potentials. Jpn J Physiol 1980; 30: 897-905.
15. Cooke KR. Ouabain and regulation of cellular volume in freshly prepared slices of rabbit renal cortex. J Physiol 1978; 279: 361-374.
16. Ayrapetyan SN, Suleymanyan MA. On The Pump-Induced Cell Volume Changes. Comp Biochem Physiol 1979; 64A: 571-575.
17. Haussinger D. Regulation of Cell Function by Hydration. Biochem J, 1996; 313: 697-710.