KETAMINE- INDUCED CELL DEHYDRATION AS A MECHANISM OF IT'S ANALGESIC AND ANESTHETIC EFFECTS

*Armenuhi Heqimyan[1], Anush Deghoyan[1], Sinerik Ayrapetyan[1]**

1. UNESCO Chair-Life Sciences International Postgraduate Educational Center, 31 Acharyan St. 0040 Yerevan, Armenia.

Abstract

Effect of intraperitoneally (i.p.) injected sub-anesthetic ($8x10^{-5}$-$8x10^{-2}$ mg/g) and anesthetic (0.125mg/g) doses of ketamine on rats' pain sensitivity and tissue hydration of different organs were studied. Determination of water content of tissue was performed by Adrian's traditional "tissue drying" experimental procedure. The number of functionally active receptors were determined by counting the number of [3H]-ouabain in tissues. Latent period of pain sensitivity was defined by means of "hot plate" test.

Ketamine in sub-anesthetic doses ($8x10^{-5}$-$8x10^{-2}$ mg/g i.p.) had depressing effect on rats' latent period of pain sensitivity which was accompanied by dehydration of tissues and decrease of the number of [3H]-ouabain receptors in membrane of tissues of different organs. The ouabain influence on brain cell hydration was characterized by dose dependent (10^{-9}-10^{-4}M) three phases and this fact was accompanied by corresponding changes of number of ouabain receptors in membrane.

Ketamine anesthetic dose had reversing effect on all three phases of ouabain – induced cell hydration. It was suggested that ketamine – induced cell dehydration leading to decrease of number of functional active proteins in membrane serves as a powerful mechanism through which an analgesic and anesthetic effects of ketamine on organisms were realized.

Article (*J Int Dent Med Res 2011; 4: (1), pp. 42-49*)

Keywords: rat, ketamine, [3H]-ouabain receptor, pain sensitivity, cell hydration.

Received date: **Accept date:**

Introduction

It is assumed that ketamine administration causes "dissociative anesthetic state" characterized by profound analgesic, moderate hypnotic properties and by marked sympathomimetic reactions.

Important adverse effects are hallucinations and hypersalivation. Pharmacological profile of ketamine influence can not be explained by a single mechanism. Analgesic, anesthetic and sympathomimetic effects are mediated by different sides of action. It is suggested that N-methyl-D-aspartate (NMDA)-receptor antagonism accounts for most of the amnestic, analgesic, psychotomimetic and neuroprotective effects of ketamine[1,2].

It is also known that ketamine blocks an NMDA receptor-mediated component of synaptic transmission in a voltage-dependent manner[3-7]. At the same time it is well established that ketamine has depressing effect on variety of receptors: nicotinic[8,9], muscarinic[10] and opioid ones[10-12] as well as on voltage sensitive Na^+ [9, 13], K^+ [13-15] and Ca^{2+} channels[16] of nerve cell membrane in peripheral and central nervous system. At present it is known that influence of ketamine is not limited only by nervous system but it has also relaxing effect on smooth and heart muscles[17-20] and other tissues[21]. However, the nature of cellular mechanism underlying in the ground of above mentioned multisided effects of ketamine on different tissues is not clear yet.

Earlier on the basis of experimental data performed *in vitro* on snail single neurons the correlation between number of functional active

Corresponding author:
Prof. Sinerik Ayrapetyan
UNESCO Chair-Life Sciences International Postgraduate Educational Center
31 Acharyan St. 0040 Yerevan, Armenia

E-mail: info@biophys.am

membrane receptors having channel[22], receptor [23], enzyme functions[24] and active cell membrane surface (cell volume) was established. According to this data cell swelling leads to the activation while shrinkage to inhibition of neurons functional activity. Therefore, the metabolic control of cell volume- induced changes of membrane functional activity was suggested as an essential mechanism through which the regulation of neuronal ionic function is realized[25].

It is known that there are a number of non-conductive membrane mechanisms responsible for cell volume regulation such as Na^+/K^+ pump, Na^+/Ca^{++} , Na^+/H^+ exchangers, changes of cytoskeleton contractility and membrane fluidity and others[26]. Previously *in vitro* experiments (on snail neurons and women breast tissue) it was shown that ketamine has stimulating effect on Na^+/K^+ pump and Na^+/Ca^+ exchanger which brings to cell dehydration[27-29].

Therefore, the working hypothesis for present work was to clarify whether the ketamine- induced cell volume changes has a crucial role in realization of analgesic and anesthetic effects as well as it's multisided effect on different organs. For this purpose the dose-dependent effect of ketamine on rats pain sensitivity, hydration of different organs tissue and the number of [^{3}H]-ouabain receptors was studied.

Materials and methods

All procedures performed on animals were carried out following the protocols approved by Animal Care and Use Committee of Life Sciences Postgraduate International Educational Centre.

Animals

All experiments were performed on 96 adult male Wistar albino rats weighing from 100g to 130g. Animals were kept in a specific pathogen-free animal room, under optimum conditions of 12 hours light/dark cycle and 22 ± 2 0C temperature, received sterilized commercial diet and *water ad libitum.*

Chemicals

Physiological solution (PS) with 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 in mM (Sigma Chemicals, Steinhein, Germany) and pH-7.4 was used in all experiments. The stock solution of ketamine containing 500mg ketamine solved in 10ml of PS was used (Gedeon Richter, Budapest, Hungary). Ketamine intraperitoneally (i.p.) injections were calculated individually according to animal body weight. [^{3}H]-ouabain (PerkinElmer, Massachusetts, USA) with 10^{-11} - 10^{-4} M concentrations were taken as a marker for definition of tissues volume changes.

Tissue preparation

Tissues of different organs (lungs, heart, spleen, liver, kidney, muscle and brain) were investigated on 50 animals. Brain tissues of next 36 animals were investigated separately. Tissues of 10 animals receiving ketamine subanesthetic dose $8x10^{-5}$ did not investigate. For definition the tissue water content and counting the number of ouabain receptors ten pieces from each organ tissue weighing from 50 to 60 mg were taken from each rat. From cortex and subcortex tissues of brain eight pieces were taken while from cerebellum tissue only four pieces for every case.

Definition of Tissues Water Content

Water content in tissues of brain and organs were determined after animals' decapitation. In definition of tissues water content 86 animals were used. Animals were sharply immobilized by dipping its' noses into liquid nitrogen for 3-4 sec[30] and decapitated after 15 min. ketamine or [^{3}H]-ouabain injection. After such procedure the full absence of somatic reflexes on extra stimuli was recorded.

Determination of water content of tissue was performed by traditional "tissue drying" method (Adrian, 1956). After measuring the tissue wet weight (w.w.) it was dried in thermostat (Factory of Medical Equipment, Odessa, Ukraine) during 24 hours at 105^0C for determination of dry weight (d. w.). The quantity of water in 1 g of d.w. of tissue was counted by the following equation: (w.w. – d.w.) / d.w. [10].

Counting the number of ouabain receptors in cell membrane

Counting the number of ouabain receptors in cell membrane was made only in samples of brain tissues (cortex, subcortex and cerebellum) in one control (18 animals) and experimental (18 animals) groups of animals. The stock [^{3}H]-ouabain solution contains 10^{-9}M concentration (12 Ci/mM specific activities). The solutions with higher concentrations (10^{-8}-10^{-4}M) were prepared by adding corresponding concentration of cold (none labeled) ouabain. Ouabain solutions were intraperitoneally injected in control as well as in

experimental groups of animals. In the last groups of animals it was made 15 minute after ketamine anesthetic dose injection. Animals were decapitated in all groups after 30 minute of injections and brain tissue samples 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 Wallac-1450 liquid scintillation counter (PerkinElmer, Finland). After this procedure the amount of scintillate isotopes counted per minute was received and correspondingly calculated as the number of ouabain receptors.

Determination of pain sensitivity latent period

This test was carried out using the specific setup developed in our laboratory. It consists of org-glass chamber with the brass bottom. The bottom temperature (51^{o}C) was controlled with the thermometer (accuracy of measurement $\pm$ 0.01^{o}C) and it was completely covered by the Plexiglas box keeping the temperature constant. Rats were placed individually on brass bottom and latent period of pain sensitivity was recorded as the time elapsed to obtain one of the following responses: licking the feet, jumping or rapidly stamping the feet. The tissue damage prevention was near 10 sec. Statistic significance in latent periods of pain sensitivity was defined between data of control and experimental groups.

Statistical Analysis

The Microsoft Excel and Sigma-Plot (Version 8.02A) were used for data analysis. For all statistical tests (with Student's t-test) P value of 0.05 or less taken as (*P < 0.05, **P < 0.01, ***P < 0.001).

Results

In first series of experiments the effect of ketamine on latent period of pain sensitivity to hot plate was investigated on four groups of animals (n = 10 in each group) injected i.p. by different subanesthetic doses ($8x10^{-5}$ - $8x10^{-2}$ mg/g) solved in 0.2 ml of PS. As a control ten rats were taken and injected i.p. with equivalent quantity (0.2 ml) of PS. Latent period of pain sensitivity was measured 15 min after injections. The mobility of animals injected with above mentioned doses of ketamine was not fully inhibited and on brass bottom of hot plate setup (t = 51^{o}) following reactions (lick the feet, jump or rapid stamp the feet) were observed in control as well as in experimental groups of animals. On Figure 1 the bars indicate in percent the value of latent period of pain sensitivity in five groups of animals. It is shown the increase of latent period of pain sensitivity more than three times after ketamine injections ($8x10^{-4}$ - $8x10^{-2}$ mg/g) and it's threshold concentration was $8x10^{-5}$ mg/g.

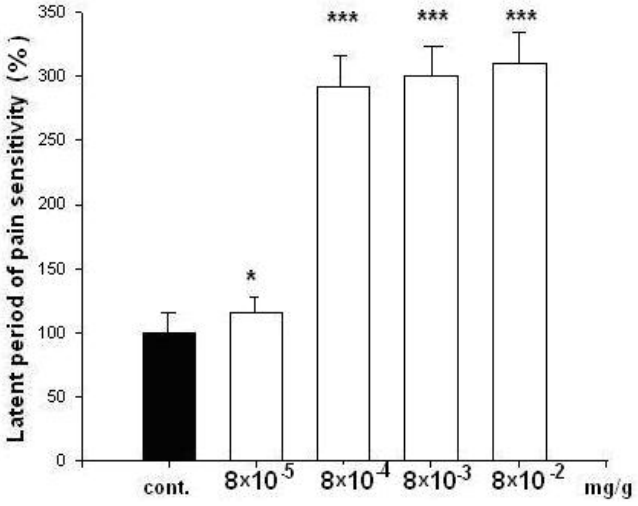

Figure 1. Ketamine effect on the latent period (in %) of pain sensitivity of rats to hot plate. Black bar indicates the latent period of pain sensitivity of control group animals injected with 0.2ml of PS. Control data was taken as 100%. White bars indicate the latent period of pain sensitivity of experimental groups' animals receiving $8x10^{-5}$ mg/g - $8x10^{-2}$ mg/g of ketamine dissolved in 0.2ml of PS. The ordinate indicates the latent period of pain sensitivity in percent (%). Each bar represents the mean of latent period from 10 animals. Error bars indicate SE. Values were statistically significant (compared with data of control group *P <0.05, ***P<0.001).

Recently in our experiments the close correlation between brain tissue hydration and pain sensitivity was demonstrated[31,32]. That is way in the second series of experiments it was interesting to clarify whether the pain relief effect of ketamine could also be due to tissue dehydration. This study was continued on previous groups of animals after determination of latent period. Experiments were performed on control and four experimental groups of animals. Ten animals for each group were chosen. Ketamine was taken i.p. in following doses: subanesthetic ($8x10^{-4}$ - $8x10^{-2}$mg/g) and anesthetic (0.125 mg/g). Dose-dependent effect of ketamine on tissue hydration of different zones

of brain (cortex, subcortex and cerebellum) and different organs were studied. Animals of control group were received 0.2 ml of PS i.p. while animals in experimental groups were injected also i.p. with different doses of ketamine: subanethetic ($8x10^{-4}$ - $8x10^{-2}$ mg/g) and anesthetic one (0.125 mg/g).

On Figure 2 dose-dependent effect of ketamine on water content (in %) in brain tissues is shown. As can be seen from Figure the curve of ketamine dose-dependent effect on cortex tissue hydration is different from those of subcortex and cerebellum. Ketamine at following concentrations ($8x10^{-4}$- $8x10^{-3}$ mg/g) has no significant effect on all brain tissues hydration while at $8x10^{-2}$ mg/g it has strong dehydration effect (decrease of water content) on all types' tissues. Ketamine at concentration of 0.125 mg/g (which had anesthetic effect on rats) shows overhydration effect (increase of water content) on cortex tissue (Figure 2, a) and dehydration effect on subcortex and cerebellum tissues (Figure 2, b,c). The fact that ketamine at anesthetic dose brings to the overhydration of cortex tissue corresponds with data where the exciting effect of ketamine on brain cortex explains by it's stimulation of some groups of glutamate receptors[33].

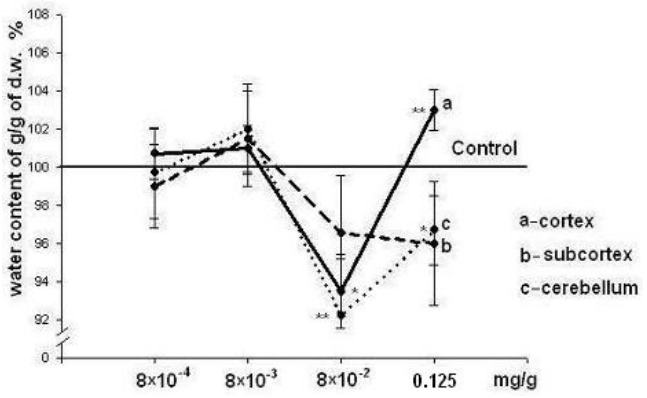

Figure 2. Dose-dependent effect of ketamine on water content in different parts of brain tissues (cortex, subcortex and cerebellum). The results are expressed in percent and control was taken as 100%. Each point is the mean value of water content for each part of brain tissue at ketamine corresponding doses (for cortex and subcortex tissues 10 samples were taken from each animal while for cerebellum tissue 4 pieces). For control and experimental groups 10 animals were chosen.

Dose-dependent effect of ketamine was also carried out on tissues of other organs (lung, heart, spleen, liver, kidney and muscle) to find out whether observed tissue hydration to ketamine is a general property or it is only specific for brain tissue.

On Figure 3 changes of tissues water content (in %) to different ketamine doses are shown. As can be seen from Figure at the smallest dose ($8x10^{-4}$ mg/g) of ketamine there is an overhydration effect in all tissues except the spleen tissue (small, no significant dehydration effect). At ketamine $8x10^{-3}$ mg/g dose in all investigated tissues dehydration effect were observed but in muscle tissue significant overhydration effect is revealed. The dehydration effect in lung, heart, spleen, kidney tissues were also found at higher doses of ketamine ($8x10^{-2}$ mg/g i.p.) but in the case of liver and muscle tissues the opposite one (overhydration effect). In the last group of animals when the anesthetic dose of ketamine (0.125 mg/g i.p.) was injected the dehydration effect on tissues of all organs except liver was observed. Thus, the presented data indicate that ketamine has modulating effect on tissue hydration of different organs and its' sensitivity to ketamine is different which can be probably explained by different nature of mechanisms involved in cell volume regulation or by various quantity of ketamine reaching these cells by blood flow.

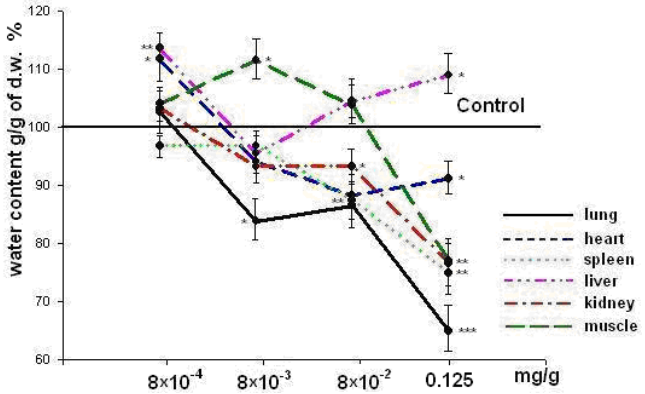

Figure 3. Dose-dependent effect of ketamine on water content in tissues of different organs. The results are expressed in percent and data of control group was taken as 100%. Each point is the mean value (n=50) of the water content for each organ of 10 animals at ketamine corresponding doses. The abscissa indicates ketamine doses - $8x10^{-4}$ mg/g - 8x10-2 mg/g and 0.125mg/g. The ordinate indicates the water

content in tissues. Error bars indicate the standard error for 10 independent experiments. Significance was calculated with Student's t test with *P < 0.05, **P< 0.01, ***P < 0.001.

Previously was shown that cell volume control is connected with Na^+/K^+ pump[24], cGMP-dependent[34] and cAMP-dependent Na^+/Ca^{++} exchangers activities[35]. In these studies by means of [^{3}H]-ouabain (specific inhibitor of Na^+/K^+ ATP-ase) cell volume changes were defined. [^{3}H]-ouabain binds to membrane Na^+/K^+ ATP-ase receptors. Kinetic curve of dose-dependent [^{3}H]-ouabain binding with neuronal membrane functionally active proteins was consisted of two saturated and one linear component. There were found that only last component of curve was connected with Na^+/K^+ pump activity[21]. From this point of view it was interesting in the last series of experiments to study the possible mechanism of ketamine anesthetic dose (0.125 mg/g) influence on dose-dependent ouabain-induced cell hydration in brain tissues and find out whether this process is accompanied with corresponding change of number of membrane functionally active proteins ([^{3}H]-ouabain was taken as a marker of membrane functionally active proteins).

These experiments were performed on 12 groups of animals (three animals in each). In first part of experiments (six groups) water content and number of ouabain receptors in brain tissues after different dose of ouabain (10^{-9}M - 10^{-6}M, 10^{-4}M) were determined. Animals of control group were injected with 0.2 ml of PS. Animals of five experimental groups were injected with different concentrations of ouabain (10^{-9}M - 10^{-6}M, 10^{-4}M) solved in 0.2ml of PS. On Figure 4 points on continuous line mean values of water content in brain tissues are shown and kinetic curve of dose-dependent ouabain effects is formed by three ranges. For ouabain 10^{-9} - 10^{-8}M concentrations an overhydration process is detected, at 10^{-7} - 10^{-6}M dose – dehydration effect and at 10^{-4}M (and less) doses again overhydration process is received. These changes of water content are characterized for all investigated tissues.

In second part of experiments (next six groups of animals) water content and number of ouabain receptors in brain tissues after ouabain and ketamine parallel injections were investigated. In this case animals of control group were injected with 10^{-9}M of ouabain solved in 0.2ml of PS. Animals of experimental groups were at first received ouabain and 15 minute after ketamine in anesthetic dose (0,125mg/g). On Figure 4 points on dotted line mean values for each group are indicated. As can be seen from figure in case of ketamine influence some reversing effect on ouabain-induced hydration of brain tissues at all doses of ouabain was observed.

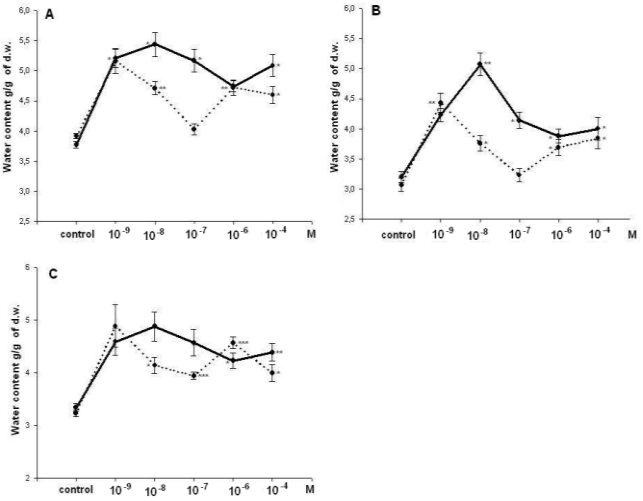

Figure 4. Dose-dependent effect of ouabain on tissues water content (brain cortex - A, subcortex -B and cerebellum - C). Continuous lines indicate the ouabain dose - dependent effect on water content in tissues of control group experiments where animals were injected with ouabain dissolved in 0,2ml PS. Dotted lines indicate the water content in tissues of experimental groups animals injected with ouabain dissolved in 0,2ml PS and 0,125mg/g ketamine. Each point on both lines shows the mean value of water content for each part of brain. Abscissae indicate control point and [3H]-ouabain concentrations (10^{-9} M - 10^{-6} M, and 10^{-4} M). Ordinates indicate the mean value of water content in tissues of cortex, subcortex and cerebellum. The difference between data of experimental and control groups of animals was statistically significant (*P<0.05, **P< 0. 01, ***P< 0.001) for all ouabain doses.

The comparison between two kinetic curves shows the following changes in cortex tissue (Figure 4 A). After 10^{-9}M of ouabain injection ketamine brings to the same overhydration effect. Ketamine-induced cell dehydration become more expressive after 10^{-8} - 10^{-7}M ouabain injection. Then, it can be noted that 10^{-6}M

ouabain dehydration effect reverses into overhydration one after ketamine influence and overhydration at 10^{-4}M ouabain also rotates into dehydration after ketamine injection.

As for subcortex tissue it can be seen (Figure 4 B) that after ketamine injections meaningful dehydration effect is observed and dotted line having same three ranges is quite parallel to continuous one.

In cerebellum tissue (after 10^{-9}M of ouabain) ketamine leads to overhydration effect more expressive than at ouabain influence (Figure 4 C). At 10^{-8} - 10^{-7}M ouabain injection ketamine shows the same dehydration effect as in case of cortex tissue. 10^{-6}M ouabain dehydration effect reverses into overhydration one after ketamine influence and overhydration (at 10^{-4}M of ouabain) to dehydration effect after ketamine injection.

The number of [3H]-ouabain receptors x10^8					
CORTEX					
	10^{-9}M	**10^{-8}M**	**10^{-7}M**	**10^{-6}M**	**10^{-4}M**
Control	0,62±0,05	1,58±0,07	2,56±0,02	3,08±0,19	5,51±0,05
Experiment	0,69±0,03	1,5±0,02	2,33±0,03	3,38±0,15	5,52±0,03
SUBCORTEX					
	10^{-9}M	**10^{-8}M**	**10^{-7}M**	**10^{-6}M**	**10^{-4}M**
Control	0,63±0,03	1,63±0,05	2,54±0,02	3,33±0,14	5,49±0,04
Experiment	0,64±0,04	1,48±0,02	2,38±0,02	3,27±0,14	5,49±0,04
CEREBELLUM					
	10^{-9}M	**10^{-8}M**	**10^{-7}M**	**10^{-6}M**	**10^{-4}M**
Control	0,48±0,08	1,58±0,08	2,65±0,05	3,48±0,05	5,43±0,07
Experiment	0,67±0,05	1,46±0,03	2,44±0,04	3,18±0,32	5,59±0,07

Table 1 - Binding of [3H]-ouabain to brain tissues (cortex, subcortex and cerebellum). As a control the number of ouabain receptors after [3H]-ouabain injections in different concentrations (10^{-9} M, 10^{-8} M, 10^{-7} M, 10^{-6} M, 10^{-4} M) was taken. In the experimental part (experiment) the number of ouabain receptors was measured after [3H]-ouabain and ketamine injections.

On table 1 the number of ouabain receptors in all investigated tissues is presented. As can be seen ketamine causes the increase of number of ouabain receptors in all three zones when injects after ouabain 10^{-9}M concentration. It is worth to note that ketamine- induced increase of number of ouabain receptors was more pronounced in cerebellum tissue than in cortex and sub-cortex tissues. At 10^{-8}M and 10^{-7}M concentrations of ouabain ketamine causes the decrease of number of ouabain receptors in all brain tissues while at ouabain 10^{-6}M concentration of this effect was observed only in subcortex and cerebellum. At 10^{-4}M concentration of ouabain ketamine has slight increasing effect on number of ouabain receptors in cortex and cerebellum tissues and no significant effect on subcortex tissues.

Discussion

Investigation of ketamine influence on rats pain sensitivity latent period, tissues hydration and number of ouabain receptors was showed that its subanesthetic doses (8x10-5-8x10-2 mg/g) have statistically significant depressing effect on rats' pain sensitivity. The analgesic effect of these ketamine doses on mammals was commonly explained by it's non-competitive inhibitory effect on NMDA receptors in brain[3-7]. However, ketamine effects on tissue hydration of various organs (lung, heart, spleen, liver, kidney and skeletal muscle) were indicated that its effect was not only connected with neuronal membrane conductive functions. This conclusion is conformed to our previous data of ketamine induced dehydration effect on women breast tissue[28].

At present it is a proven fact that metabolic control of cell volume is dynamic cell parameter through which the regulation of cell various functions is realized[25,26,36,37]. Non-linear curves of dose-dependent ketamine effect on tissue hydration in brain and different organs could be explained by multisided effects of ketamine on mechanisms involved in cell volume regulation.

Although there is a number of metabolic mechanisms involved in cell volume regulation the electrogenic ionic transporting mechanisms such as Na^+/K^+ pump and Na^+/Ca^{++} exchanger have a crucial role in this process[38-40]. Earlier it was shown that ketamine has activation effect on electrogenic Na^+/K^+ pump and Na^+/Ca^{++} exchanger activity in isolated neurons of mollusc through which it causes cell dehydration and decrease of membrane fluidity[24,41].

In study of dose-dependent ouabain binding with neuronal membrane three types of ouabain receptors was distinguished[24]. Among them the function of only one-dose dependent linear binding having low agonist affinity connected with Na^+/K^+ pump inhibition while the function of two types receptors having higher agonist affinity and characterized by dose-dependent saturated curves connected with cGMP-dependent Na^+/Ca^{++} and cAMP-dependent Na^+/Ca^{++} exchangers mechanisms

correspondingly[24,34,35]. As these ion transporting mechanisms are electrogenic one they could modulate the level of cell hydration: activation of cGMP-dependent Na^+/Ca^{++} exchanger and inactivation of Na^+/K^+ pump brings to cell swelling while activation of cAMP-dependent Na^+/Ca^{++} exchanger brings to cell shrinkage.

Considering the curves of dose-dependent ouabain-induced tissue hydration (Figure 4), it can be clearly seen three phases of ouabain-induced changes on tissue hydration: overhydration at 10^{-9}-10^{-8}M, dehydration at 10^{-7}-10^{-6}M and overhydration at 10^{-4}M. The fact that the ketamine has reverse effects on all the three components of ouabain-induced hydration brings to conclusion that above mentioned ouabain receptors function which connected with cGMP-dependent Na^+/Ca^{++}, cAMP-dependent Na^+/Ca^{++} exchangers and Na^+/K^+ pump activity are ketamine sensitive. This conclusion confirms the previous isotope study of ketamine effects on Na^+/K^++ pump activity and Na^+/Ca^{++} exchanger in snail neurons according which ketamine has activation effect on Na^+/Ca^{++} (Ca^{++} efflux Na^+ influx) exchange and Na^+/K^+ pump activity (24).

The fact that ketamine has slight hydration effect on brain tissues which was accompanied by increased number of ouabain receptors at 10^{-9}M ouabain concentration (Table 1) could be explained by the activation of cGMP dependent Na^+/Ca^{++} exchanger which is responsible for cell hydration as it was above mentioned.

Non-anesthetic dose (8x10-2mg/g i.p.) of ketamine has dehydration effect on brain tissues which is accompanied by corresponding decrease of number of ouabain receptors at 10^{-8}-10^{-7} M ouabain concentrations (Table 1) explained by activation of cAMP dependent Na^+/Ca^{++} exchanger (Ca^{++}influx and $3Na^+$ efflux) responsible for cell dehydration[34,35].

Thus, the above mentioned results and literature data about involvement of intracellular cyclic nucleotides in brain psychological processes[42] leads to the hypothesis that hallucination effect of ketamine connected with its modulation effect on cyclic nucleotides metabolism which could serve as a subject for the future investigation.

The data of close correlation between cell hydration and the number of functional active protein molecules determining the cell functional activity[25] and recent data on direct correlation between brain tissue hydration and rats pain sensitivity[32] allows to consider ketamine dehydration effect at sub-anesthetic doses as an essential mechanism responsible for its depression effect on pain sensitivity of rats. On basis of the fact that ketamine anesthetic dose has only dehydration effect on subcortex and cerebellum tissue and over hydration effect on cortex tissue the suggestion can made that anesthetic effect of ketamine is only responsible for subcortex and cerebellum tissues. This conclusion can not be final as it needs future investigation.

Conclusions

Obtained data allow to decide that analgesic effect of subanesthetic dose of ketamine can be explained by brain tissue dehydration. Dehydration effect in tissues of subcortex and cerebellum has essential role in realization of ketamine anesthetic effect on organism. Ketamine induced cell volume changes accompanied by corresponding changes of number of functional active proteins on cell membrane serve as one of the main nonconductive membrane mechanisms through which the biological effect of ketamine is realized and explanation of ketamine effects only by changes of neuronal membrane conductive functions is not adequate.

Acknowledgements

The authors thank to Mss. Anna Nikoghosyan and Varsik Martirosyan for technical and editorial assistance.

Declaration of Interest

The authors report no conflict of interest and the article is not funded or supported by any research grant.

References

1. Kress HG. Wirkmechanismen von Ketamin. Anaesthesist 1997; 46: S8-s19.
2. Kohrs R, Durieux ME. Ketamine: teaching and old drug new tricks. Anesth.Analg 1998; 87: 1186-1193.
3. Davies SN, Alford ST, Coan EJ, Lester RA, Collingridge GL. Ketamine blocks an NMDA receptor- mediated component of synaptic transmission in rat hippocampus in a voltage-dependent manner. Neurosci Let 1988; 92: 213-7.
4. Reich DL, Silvay G. Ketamine: an update of the first twenty-five years of clinical experience. Canad. J. of Anaesthesa 1989; 36: 186-197.

5. Franks NP, Lieb WR. Molecular and cellular mechanisms of general anaesthesia. Nature 1994; 367: 607–614.
6. Sakai F, Amaha K. Midazolam and ketamine inhibit glutamate release via a cloned human brain glutamate transporter. Canad. J. of Anesthesia 2000; 47: 800-6.
7. Franks NP. General anesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nature Reviews (Neuroscience) 2008; 9: 370-386.
8. Scheller M, Bufler J, Hertle I, et al. Ketamine blocks currents through mammalian nicotinic acetylcholine receptor channels by interaction with both the open and the closed state. Anesth. Analg 1996; 83: 830–6.
9. Sasaki T, Tomio A, Itaru W, et al. Nonstereoselective inhibition of neuronal nicotinic acetylcholine receptors by ketamine isomers. Anesth Analg 2000; 91: 741–8.
10. Hustveit O, Maurset A, Oye I. Interaction of the chiral forms of ketamine with opioid, phencyclidine, sigma and muscarinic receptors. Pharmacol. Toxicol. 1995; 77: 355–9.
11. Finck AD, Ngai SH. Opiate receptor mediation of ketamine analgesia. Anesthesiology 1982; 56: 291–297.
12. Smith DJ, Bouchal RL, Desanctis CA, et al. Properties of the interaction between ketamine and opiate binding sites in vivo and in vitro. Neuropharmacology 1987; 26: 1253–1260.
13. Schnoebel R, Wolff M, Peters SC, et al. Ketamine impairs excitability in superficial dorsal horn neurons by blocking sodium and voltage-gated potassium currents. Brit J. of Pharmacology 2005; 146: 826–833.
14. Brau ME, Sander F, Vogel W, Hempelmann G. Blocking mechanisms of ketamine and its enantiomers in enzymatically demyelinated peripheral nerve as revealed by single-channel experiments. Anesthesiology 1997; 86: 394–404.
15. Kawano TK, Oshita S, Takahashi A, et al. Molecular Mechanisms Underlying Ketamine-mediated Inhibition of Sarcolemmal Adenosine Triphosphate-sensitive Potassium Channels. Anesthesiology 2005; 102: 93-101.
16. Hirota K, Lambert DG. Ketamine: its mechanism(s) of action and unusual clinical uses. Brit. J. of Anaesthesia 1996; 77: 441-4.
17. Akbaş L., Sarioğlu Y., Utkan T. Effect of ketamine on contractile performance of isolated frog myocardium and comparison of ketamine, thiopental and droperidol. Mater Med Pol. 1992;24 (1): 32-4
18. Abdalla SS, Laravuso RB, Will JA. Mechanisms of the Inhibitory Effect of Ketamine on Guinea Pig Isolated Main Pulmonary Artery. Anesth. Analg. 1994; 78: 17-22.
19. Kohjitani A, Shirakawa J, Okada S, Obara H. The Relaxing Effect of Ketamine on Isolated Rabbit Lower Esophageal Sphincter. Anesth Analg. 1997; 84: 433-7.
20. Han J, Kim N, Joo H, Kim E. Ketamine blocks Ca2+-activated K+ channels in rabbit cerebral arterial smooth muscle cells. Am J Physiol Heart Circ Physiol. 2003; 285: 1347-1355.
21. Chang Y, Chen TL, Wu GJ, et al. Mechanisms involved in the antiplatelet activity of ketamine in human platelets. Journal of Biomedical Science 2004; 11: 764-772.
22. 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: 179-186.
23. Ayrapetyan SN, Arvanov VL. On the mechanism of the electrogenic sodium pump dependence of membrane chemosensitivity. Comp Biochem Physiol 1979; 64: 601-604.
24. Ayrapetyan SN, Suleymanyan MA, Sagian AA, Dadalyan SS. Autoregulation of Electrogenic Sodium Pump. Cell Mol Neurobiol. 1984; 4: 367- 384.
25. Ayrapetyan SN. On the physiological significance of pump-induced cell volume changes. Adv. Physiol. Sci. 1980; 23: 67-82.
26. Hoffman EK, Lambert IH, Pedersen SF. Physiology of cell volume regulation in vertebrates. Physiol. Rev. 2009; 89:193-277.
27. Ayrapetyan SN, Takenaka MA, Bakunts IS, Saghyan AA, Dadalyan SS. Reports of USSR Academy of Sciences 1986; 291:469-472.
28. Danielyan AA, Mirakian MM, Airapetyan SN. The dehydrating action of ketamine on malignant breast tumors. Voprosi Onkol. 1998; 44: 395-7.
29. Danielyan AA, Ayrapetian SN. Changes of hydration of rats' tissues after in vivo exposure to 0.2 Tesla Steady Magnetic Field. Bioelectromagnetics 1999; 20: 123-128.
30. Takahashi R, Aprison MH. Acetylcholine content of discrete areas of the brain obtained by a near-freezing method. J. of Neurochemistry 1964; 11:887-892.
31. Adrian RH. The effect of internal and external potassium concentration on the membrane potential of frog muscle. J Physiol 1956; 133: 631–658.
32. Ayrapetyan S, Musheghyan G, Deghoyan A. The brain tissue dehydration as a mechanism of analgesic effect of hypertonic physiological solution in rats. J Int Dent Med Res 2010; 3: 93-9.
33. Lazzaro V, Oliviero A, Profice P, et al. Ketamine increases motor cortex excitability to transcranial magnetic stimulation Physiol. 2003; 547: 485–496.
34. Azatian KV, White AR, Walker RJ, Ayrapetyan SN. Cellular and molecular mechanisms of nitric oxide-induced heart muscle relaxation. Gen. Pharmac. 1998; 30:543-553.
35. Saghyan AA, Hunanian Ash, Ayrapetyan SN. Effect of Locust Poison (LP) on Neuromembrane Functional Activity. General Pharmacology 1997; 29:587-590.
36. Tasaki I, Iwasa K. Rapid pressure changes and surface displacements in the squid giant axon associated with production of action potentials. Jpn J Physiol. 1982; 32:69-81.
37. Haussinger D. Regulation of Cell Function by Hydration, Biochem. J. 1996; 313:697-710.
38. Cooke KR. Ouabain and regulation of cellular volume in freshly prepared slices ofrabbit renal cortex. J Physiol. 1978; 279:361-374.
39. Ayrapetyan SN, Suleymanyan MA. On The Pump-Induced Cell Volume Changes. Comp Biochem Physiol 1979; 64: 571-5.
40. Annunziato L, Pignataro G, Di Renzo GF. Pharmacology of Brain Na_/Ca2_ Exchanger: From Molecular Biology to Therapeutic Perspectives Pharmacol Rev 2004; 56: 633–654.
41. Saghyan AA, Dadalian SS, Takenaka T, Suleymanyan MA, Ayrapetyan SN. The effects of short-chain fatty acids on the neuronal membrane functions of helix pomatia. III 22 Na efflux from the cells. Cell. And Mol. Neourobiol. 1986; 6: 397-405.
42. Kandel ER, Schwartz JH. Molecular biology of learning: modulation of transmitter release. Science 1982; 218: 433-442.