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IN VIVO AND *IN VITRO* EFFECTS OF HYPERGLYCEMIA ON Na^+-K^+, Ca^{+2}, Mg^{+2}- DEPENDENT ATPases ACTIVITY IN BRAIN SYNAPTOSOMES OF AGING RATS

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Cerebral metabolism of glucose, one of the determinants of tissue ATP level, is crucial for the CNS function. The activity of P-type pumps: Na^+, K^+-ATPase, Ca^{+2}-ATPase and Mg^{+2}-ATPase were examined in rat brain synaptosomes to determine if changes in the enzyme activity related to aging are potentially associated with alterations in glucose homeostasis. Male Wistar rats (newborn, 3- and 18-month-old) were sacrificed by decapitation and synaptic plasma membranes were isolated from brains. *In vivo* study demonstrated that 18-month-old rats were characterized by hyperglycemia, hyperinsulinemia and increased total antyoxidative status (TAS) level. These conditions had a different impact on activities of the ATPases tested *in vivo*: only the activity of Ca^{+2}-ATPase decreased whereas that of Mg^{+2}-ATPase increased significantly. *In vitro* experiments, prior incubation of isolated synaptosomes with glucose of concentrations corresponding to normoglycemia *in vivo* (4.5 - 6.5 mM), stimulated Ca^{+2}-ATPase activity, whereas higher glucose concentrations (10.0 - 12.5 mM) inhibited significantly the enzyme activity. The most sensitive to hyperglycemia appeared Na^+, K^+-ATPase in old rats synaptosomes with the progressive decline starting at 6.5 mM glucose. The activity of Mg^{+2}-ATPase was not inhibited *in vitro* even at high glucose concentrations that may explain the increased *in vivo*, activity of this enzyme in old, hyperglycemic rats.

Key words: *Na^+, K^+-ATPase, Ca^{+2}-ATPase, Mg^{+2}-ATPase, brain synaptosomes, hyperglycemia, aging.*

INTRODUCTION

Normal function of the nervous system *in vivo* depends on substrates supplied by the blood, among them glucose is considered essential for physiological behavior of the CNS. The energy metabolism of the brain and the blood flow that sustains it vary considerably from birth to old age, that was proved on the basis of the results of *in vitro* measurements in animal brain preparations and in intact animals. Research in human patients studied with the [^{18}F]-fluorodeoxyglucose technique and PET demonstrate a similar pattern of rises in cerebral glucose utilization from early life to a peak around the time of puberty and then a decline from that peak down to normal adult levels (1). The effects of aging on cerebral circulation and energy metabolism are difficult to separate from those of diseases, mainly vascular, that are almost inextricably intertwined with the aging process. Insulin resistance in old, compared with young, humans and animals has been also documented to lead finally to hyperglycemia and hyperinsulinemia. However, in some conditions hyperglycemia can be beneficial for the organism. According to the results of Ekholm *et al.* (2), hyperglycemia retards the loss of ion homeostasis generating production of additional ATP which in turn supports Na^+, K^+-driven ATPase activity during ischemia.

The glucose transporters present in the brain tissue have a relatively low affinity to the substrate (high K_m value of 7-11 mmol/l) although the K_m value is of the same order of magnitude as the concentration of glucose in plasma. Thus the affinity is matched to the actual concentration which allows a sufficient binding of glucose to the carriers, namely GLUT-1 and GLUT-3. As the result of cerebral glucose metabolism, concentration of intracellular ATP generated in brain tissue amounts to 3.0 - 3.5 mM, whereas ATP levels in other tissues may vary from 2.5 to 6 mM. Energy derived from ATP breakdown is needed for the activity of plasma membrane ATPases, therefore any fluctuations in glucose concentrations, which are able to modify intracellular ATP level, have to affect the activity of P-type pumps, e.g. Na^+, K^+-ATPase in the nervous tissue. The activity of this enzyme has been found to increase in developing rat brain and to decrease during aging (3). A study on the effect of aging on Ca^{+2}-ATPase activity in rat brain synaptosomal fractions revealed a progressive age-dependent decrease of the enzyme activity (4). In our previous study we have shown that 18-month-old rats are characterized by hyperglycemia, hyperinsulinemia and greatly elevated insulin/glucose ratio, the indicator of insulin resistance. These alterations in glucose homeostasis had a different impact on activities of the ATPases tested in rat brain synaptosomes: a significant decrease was observed only in Ca^{+2}-ATPase activity whereas the activity of Mg^{+2}-ATPase increased significantly (5).

Thus the aim of our present study is to elucidate if the changes in the activity of Na^+, K^+-ATPase, Ca^{+2}-ATPase and Mg^{+2}-ATPase in rat brain synaptosomes are due to hyperglycemia both *in vivo* and *in vitro* conditions. Our review of the literature revealed no comparative study on the activity of all these three ATPases

tested in synaptosomes isolated from normo- and hyperglycemic rats *in vitro* conditions with different concentrations of exogenous glucose corresponding to endogenous blood glucose concentrations in rats of different age.

MATERIAL AND METHODS

The experiments were performed on male Wistar rats and conducted following the experimental protocol approved by the Committee for Research and Animal Ethics of the University of Medical Sciences in Poznań. The animals were housed in cages at normal room temperature and maintained on standard laboratory chow (LSM) with free access to food and water. All the experiments were carried out between 9-11 a.m. The rats were sacrificed by decapitation and after complete exsanguination brains were removed immediately and used for isolation of synaptosomes. In the present study brain synaptosomes were isolated from three groups of rats: newborn (5-day-old, weighing 5.8 - 6.0 g), young (3-month-old, weighing 220 - 250 g) and old (18-month, weighing 595 - 630 g) animals.

The tissue was homogenized in 20 vol of 0.32 M sucrose at 4°C.

In vivo experiments

Preparation of synaptosomes

Synaptosomes were isolated from rat brains by the method of Lin and Way (6, 7), as described previously (5). Briefly, a whole-brain homogenates in 0.32 M sucrose - 5 mM HEPES buffer, pH 7.5, were centrifuged at 1000 x g for 10 min. The resultant supernatant was centrifuged for 30 minutes at 17 000 x g to obtain the crude synaptosomal fraction. The fraction washed twice and suspended in 0.32 M sucrose HEPES-buffer was subjected to Ficoll gradient centrifugation (63 000 x g for 1 hour). Synaptosomes collected at the interfaces between Ficoll layers (7.5% and 12% in sucrose - HEPES solution) were pelleted and suspended in 0.32 M sucrose solution for enzyme assays within 2 hours. All the procedures described above were carried out at 4°C.

ATPases assay

The Na^+, K^+-ATPase activity was estimated by the method of Muszbek *et al.* (8). The enzyme activity was determined by measuring the amount of inorganic phosphate (P_i) liberated from ATP during the incubation of synaptosomal fraction. The reaction mixture contained 100 mM NaCl, 20 mM KCl, 2mM ATP (disodium salt), 30mM Tris-HCl buffer (pH 7.4) and the synaptosomes (50 µg of protein) in a final volume of 1 ml. After a 10-min. preincubation at 37°C in the presence of 1.5 mM ouabain to specifically inhibit Na^+, K^+- ATPase, the reaction was initiated by addition of ATP, and terminated after 15 min. incubation by addition of 500 µl of 15% (w/v) trichloroacetic acid. The released inorganic phosphate was assayed by the spectrophotometric method of Goldberg (9). Na^+, K^+-ATPase activity was calculated from the difference between amounts of inorganic phosphate found after incubation in the absence and presence of 1.5 M ouabain.

The Mg^{+2}-ATPase activity and Ca^{+2}-ATPase activity were measured by the method of Lin and Way (7). The assay medium (1 ml) contained 50 mM imidazole-HCl buffer (pH 7.5), 0.4 mM $MgCl_2$ or 0.4 mM $CaCl_2$, 2 mM ATP and the synaptosomal fraction (50 µg protein) suspended in 0.32 M sucrose. After 15 minutes of incubation at 37°C the reaction was stopped by adding 1.2 ml of ice cold 10% TCA. The Ca^{+2}-ATPase (or Mg^{+2}-ATPase) activity represented the difference between the enzyme activity in the presence and absence of Ca^{+2} (or Mg^{+2}) cations.

Activities of all the ATPases tested, were expressed in μmol P_i liberated from ATP by 1 mg of synaptosome protein during one hour (μmol $P_i \times hour^{-1} \times mg\ protein^{-1}$). Protein concentration was assayed by the method of Lowry *et al.* (10) using bovine serum albumin as a standard.

Blood analysis

Serum insulin was measured by a standard radioimmunoassay (RIA) using kits for rat insulin estimation (Linco, Research Inc. USA). Glucose was determined by the glucose oxidase method (Sigma). TAS (Total Antyoxidative Status) was estimated by the colorimetric method using a standard kit produced by Randox Laboratories Ltd (United Kingdom).

In vitro experiments

For the *in vitro* experiments synaptosomes from brains of 5-day-old, 3-month-old and 18-month-old rats were isolated by the same method as described above. Immediately after the whole procedure of isolation, synaptosomes obtained from this three groups of rats were preincubated for 1 hour (30 mM Tris - HCl buffer pH 7.4) at different concentration of glucose corresponding either to normoglycemia *in vivo* (4.5 mM and 6.5 mM) or to hyperglycemia of the order of magnitude found in our hyperglycemic, old rats *in vivo* (10.0 mM and 12.5 mM). After preincubation of synaptosomes of each group of the rats in normo- and hyperglycemic condition the ATPases assays was performed by the method of Muszbek as described previously.

Statistical analysis was performed using nonparametric Mann-Whitney test. Differences were considered to be significant at a level of $p<0.05$.

RESULTS

In vivo experiments

Blood analysis

The results summarized in *Fig. 1* demonstrate that 18-month-old rats are characterized by hyperglycemia and hyperinsulinemia. In the group of newborn rats (5-day-old) serum glucose concentration was found to be 4.35 ± 0.50 mmol/l whereas in the group of young, mature rats (3-month-old) and 18-month-old rats amounted to 6.32 ± 0.30 mmol/l and 10.26 ± 0.30 mmol/l, respectively. The highest glucose concentration observed in the oldest rats was significantly elevated as compared to both newborn and young rats ($p < 0.01$).

Insulin concentration in blood serum of the 18-month-old rats was enormously increased (114.0 ± 5.59 μU/ml) in comparison to insulin levels measured in serum of newborn (14.88 ± 3.71 μU/ml) and young, mature rats (19.00 ± 1.91 μU/ml). When values of TAS (Total Antyoxidative Status) estimated in young, mature rats, commonly used as experimental animals, were accepted as 100% (*Fig. 1-C*) it was found that levels of TAS in both 5-day-old and 18-month-old rats were significantly higher ($p < 0.01$). Interestingly, TAS level in 18-month-old rats appeared the highest one, probably as a result of compensatory mechanisms for hyperglycemia (the primer of oxidative stress).

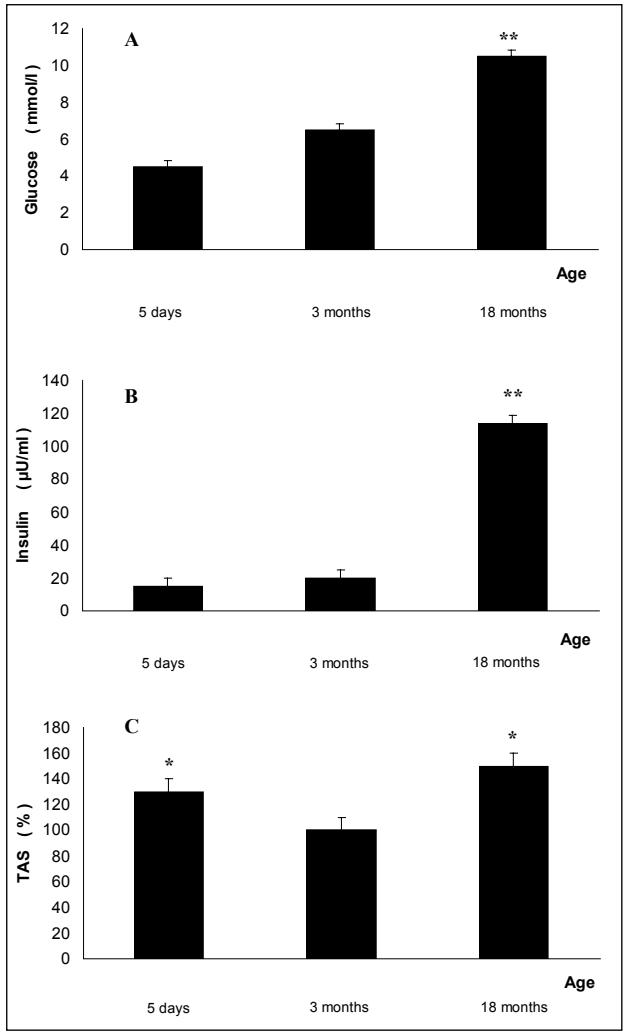

Fig. 1. Some blood parameters in 5-day-old, 3-month-old and 18-month-old rats.
(A). Serum concentrations of glucose expressed in nmol/l. Results are presented as mean ± SEM (n=10). Asteriks (**) indicate that changes are significantly different ($p< 0.01$) from both newborn (5 days) and young, mature rats (3 months)
(B). Plasma concentrations of immunoreactive insulin expressed in µU/ml. Results are presented as mean ± SEM (n= 10). Asteriks (**) indicate that changes are significantly different ($p < 0.01$) from both newborn (5 days) and young, mature rats (3 months)
(C). Percentage changes in TAS (Total Antioxidative Status) expressed in % of control level. Values obtained from mature rats (3-month-old) were accepted as control (100%). Asteriks (*) indicates that changes are significantly different ($p < 0.01$) from both newborn (5 days) and old (18 months) rats.

Na^+, K^+, Ca^{+2}, Mg^{+2}-dependent ATPases assays

Measurements of Na^+, K^+-ATPase, Ca^{+2}-ATPase, and Mg^{+2}-ATPase activities were carried out as described in Methods. Our studies were performed using synaptosomes isolated from brains of male rats that were either 5-day-old (newborn) or 3-month-old (young) and 18-month-old (aging). As shown in *Fig. 2* Na^+, K^+-ATPase activity, expressed in µmol $P_i \times min^{-1} \times$ mg $protein^{-1}$ was increased in synaptosomes isolated from both young (0.30 ± 0.021) and old rats (0.20 ± 0.020) as compared to newborn rats (0.11 ± 0.044). However, a drop in activity of this enzyme in synaptosomes of old rats as compared to the activity found in synaptosomes of young, 3-month- old rats was not significantly different at $p < 0.01$.

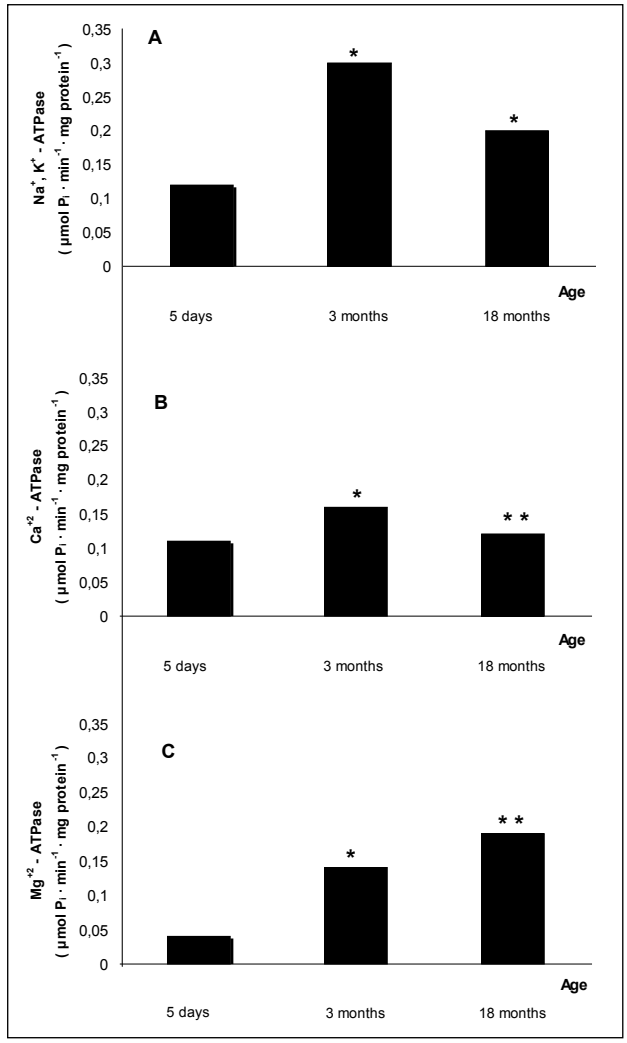

Fig. 2. *In vivo* activities of Na^{+}, K^{+}-ATPase (A), Ca^{+2}-ATPase (B) and Mg^{+2}-ATPase (C) in brain synaptosomes of newborn (5 days), young (3 months) and old rats (18 months). All the activities are expressed in µmol $P_i \times min^{-1} \times$ mg $protein^{-1}$. Asteriks (*) indicates changes statistically different ($p < 0.01$) from newborn rats, whereas (**) marks changes significantly different ($p < 0.01$) from activities found in both newborn and young rats.

Ca^{+2}-ATPase activity was also the highest one in group of 3-month-old rats (0.16 ± 0.013 µmol $P_i \times min^{-1} \times$ mg $protein^{-1}$) as compared both to the newborn rats (0.11 µmol $P_i \times min^{-1} \times$ mg $protein^{-1}$) and the 18-month-old rats (0.12 ± 0.010 µmol $P_i \times min^{-1} \times$ mg $protein^{-1}$). The mentioned above changes were significant ($p < 0.01$). As shown in the same *Fig. 2*, Mg^{+2}-ATPase activity progressively increased with the increasing age of animals. The enzyme activity (expressed in µmol $P_i \times min^{-1} \times$ mg $protein^{-1}$) increased from 0.04 ± 0.011 in synaptosomes isolated from newborn rats to 0.14 ± 0.015 in 3-month-old rats and further up to 0.019 ± 0.013 in synaptosomes of 18-month-old animals. The highest activity of Mg^{+2}-ATPase was observed in the brain synaptosomes of the oldest rats and this

increase in the enzyme activity was significant as compared to both newborn and young rats ($p < 0.01$). In conclusion, activities of Na^+, K^+-ATPase, Ca^{+2}-ATPase and Mg^{+2}-ATPase in synaptosomes obtained from brains of newborn, young and old rats differ among group tested. The activities of Na^+,K^+-ATPase and Ca^{+2}-ATPase were found to be the highest in brain synaptosomes isolated from young, mature rats (3-month-old) whereas surprisingly, the activity of Mg^{+2}-ATPase was significantly elevated in the group of 18-month-old rats.

In vitro experiments

After the isolation procedure, brain synaptosomes were preincubated at different concentrations of glucose corresponding either to normoglycemia *in vivo* (4.5 mM and 6.5 mM) or to hyperglycemia *in vivo* (10.0 - 12.5 mM). Then the activity of these all three ATPases tested was measured by the same methods as *in vivo* study.

As shown in *Fig. 3*, Na^+-K^+-ATPase activity was the highest one in synaptosomes isolated from brains of 3-month-old rats, however, there were no significant changes between the enzyme activity at different glucose concentrations (4.5 mM, 6.5 mM, 10.0 mM and 12.5 mM). Na^+, K^+-ATPase activity ranged between 3.2 ± 0.82 and 4.0 ± 1.14 μmol $P_i \times hour^{-1} \times mg\ protein^{-1}$.

In synaptosomes isolated from brains of newborn rats activity of Na^+, K^+-ATPase was relatively low ranging from 0.83 ± 0.08 at glucose concentration of 4.5 mM in incubation medium to 0.41 ± 0.05 μmol $P_i \times hour^{-1} \times mg\ protein^{-1}$ at glucose concentration of 12.5 mM. As for Na^+, K^+-ATPase measured in synaptosomes of 18-month-old rats, its activity ranged between 3.2 ± 1.05 and 0.57 ± 0.12 μmol $P_i \times hour^{-1} \times mg\ protein^{-1}$. Interestingly, the highest activity of this enzyme was observed at glucose concentration of 4.5 mM in incubation medium and then a progressive decline in activity was seen with increasing glucose concentrations. The lowest value of this enzyme activity was found at the highest glucose concentration tested (12.5 mM). These data indicate that Na^+, K^+-ATPase activity in synaptosomes obtained from 18-month-old rats characterized by hyperglycemia is the most sensitive to inhibitory glucose effect *in vitro*.

By contrast, activity of Mg^{+2}-ATPase was the highest one in synaptosomes isolated from newborn rats. It ranged between 1.6 ± 0.36 and 3.4 ± 0.72 μmol $P_i \times hour^{-1} \times mg\ protein^{-1}$ being stimulated by increasing glucose concentrations. In synaptosomes obtained from 3-month-old rats the highest activity of Mg^{+2}-ATPase was observed at glucose concentration of 6.5 mM, whereas further increase in glucose concentration in incubation medium inhibited the enzyme activity. On the contrary, activity of Mg^{+2}-ATPase in synaptosomes of 18-month-old rats was rather low, ranging from 0.51 ± 0.19 to 0.64 ± 0.10 μmol $P_i \times hour^{-1} \times mg\ protein^{-1}$ but was not inhibited by increasing glucose concentrations in incubation medium.

As it concerns Ca^{+2}-ATPase activity, it was higher at lower glucose concentrations of 4.5 and 6.5 mM but at glucose concentrations of 10.5 mM and

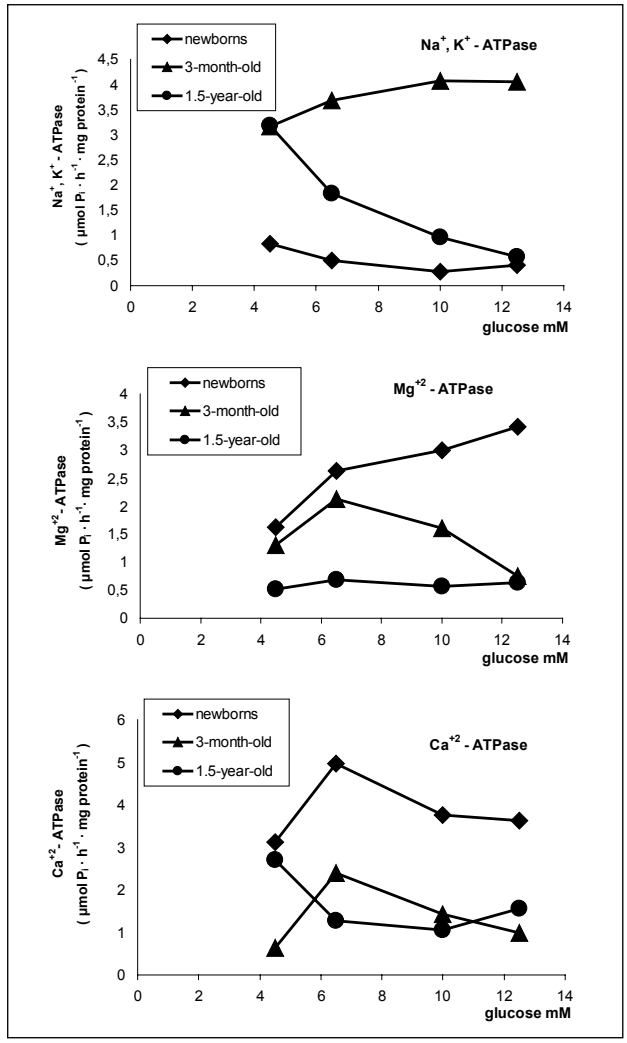

Fig. 3. In vitro activities of Na^+, K^+-, Mg^{+2}-, and Ca^{+2}-ATPases in synaptosomes isolated from brains of newborns (5 days), 3-month-old and 18-month-old rats. After isolation procedures synaptosomes were preincubated during 1 hour at different glucose concentrations. The activity of the ATPases tested were measured by the same method as *in vivo* study but expressed in µmol $P_i \times min^{-1} \times$ mg $protein^{-1}$.

12 mM in incubation medium its activity decreased. This dose - dependent effect of glucose was observed in synaptosomes of the all groups of rats.

Thus it may be concluded, that *in vitro* conditions the most sensitive to inhibitory effect of glucose appeared Na^+, K^+-ATPase in synaptosomes isolated from 18-month-old rats. On the other hand, stimulatory effect of glucose, especially at higher glucose concentration of 10.5 and 12.0 mM was observed in the case of Mg^{+2}-ATPase in synaptosomes isolated from newborn, 5-day-old rats. Moreover, the activity of Mg^{+2}-ATPase in synaptosomes of 18-month-old rats was not inhibited by any glucose concentrations tested that is consistent with *in vivo*

results that revealed increased activity of this enzyme in synaptosomes of the oldest, hyperglycemic rats.

DISCUSSION

It has been shown that both acute (11) and chronic (12) hyperglycemia modify the oxidative status of nervous tissue. Enhanced oxidative stress and changes in antioxidant balance, which lead to the release of excitatory neurotransmitters and disruption of ionic homeostasis, have been observed in both experimental and clinical diabetes (13).

Na^+, K^+-ATPase (E.C.3.6.1.3) is a membrane integral enzyme responsible for the pumping functions that is essential in restoring ion-gradients across plasma membranes in electrically excitable tissues. This enzyme activity has been proposed to be used as a potential indicator for membrane structure and function (14).

The inactivation of P-type pumps, e.g. Na^+, K^+-ATPase leads to partial membrane depolarization allowing excessive Ca^{+2} entry inside neurons with resultant excitotoxic events. This membrane bound enzyme requires phospholipids for its activity and is highly vulnerable to oxidative damage evoked by reactive oxygen radicals or lipid peroxidation products.

Hyperglycemia is the primer of a series of cascade reactions causing overproduction of free radicals that leads finally to damage of enzyme protein. As shown by Aragno *et al.* (15), the hyperglycemia - induced damage of the central nervous system is brought about by a detrimental effect of chronic hyperglycemia on the integrity of synaptic membranes due to overproduction of free radicals.

Another pathway, in addition to generation of free radicals that is involved in age-related cellular degeneration is the accumulation of advanced glycosylation end-products (AGE). Under *in vivo* conditions AGE formation depends mainly on the blood glucose concentration that has been proved to be elevated with age. There is growing number of evidences showing that hyperglycemia may inhibit the activity of several enzymes due to enzyme protein glycation (16, 17).

Our experiments were carried out in synaptosomes isolated from brains of male Wistar rats that were 5-day-old (newborns), 3-month-old (young) and 18-month-old (aging). As shown by Fraser *et al.* (18, 19) activities of Na^+, K^+-ATPase in rat brain synaptosomes are greater in males than in female rats, and these differences are even more significant in aging rats (12- and 19-month-old). The results of the mentioned studies suggest that the Na^+, K^+-ATPase pump function is significantly decreased in female rats with advancing age whereas the Na^+, K^+-ATPase activity in male rats does not decrease during aging. It is unclear why this differences occurs between sexes, but the availability of circulating testosterone in males until old age may contribute to the observed phenomenon. Taking into consideration gender differences in this enzyme activity, probably due to lack or abundance of reproductive hormones, we decided to isolate

synaptosomes only from the brains of male rats. This is the reason for which the activity of Na^+, K^+-ATPase estimated by us is of the same order of magnitude as the activity measured by Fraser (19). For example, activity of Na^+, K^+-ATPase in brain synaptosomes of our 18-month-old rats equals 0.225 ± 0.020 whereas in 19-month-old rats tested by these authors activity of this enzyme is found to be 0.274 ± 0.017 (as calculated in μmol $P_i \times min^{-1} \times$ mg $protein^{-1}$).

In the present *in vivo* experiments we have shown that hyperglycemia and hyperinsulinemia occurring in the old rats, had a different impact on activities of the ATPases tested. Na^+, K^+-ATPase activity in male rats synaptosomes remains almost unchanged with age that is consistent with Fraser study. Surprisingly, blood analysis revealed that TAS level in old rats was elevated as compared to the values obtained from both newborn and young, mature rats. The possibility that mild hyperglycemia, that is not able to affect the Na^+, K^+-ATPase activity significantly, makes organism more resistant to oxidative stress might be considered. It has been reported that streptozocin-induced diabetes protects stroke-prone spontaneous hypertensive rats against stroke (20) and that hyperglycemia preserves the brain oxydative phosphorylation during ischemia better than normoglycemia does (21). These findings lead to hypothesis that hyperglycemia provides sufficient substrate to prolong cellular energy metabolism, supporting Na^+, K^+-ATPase pumping activity. Moreover, it has also been shown that marked hyperglycemia induced by the administration of 2-DG to satiated rats is associated with significant increase in Na^+, K^+-ATPase activity (22).

By contrast, activity Ca^{+2}-ATPase in brain synaptosomes of old rats decreases significantly as compared to young mature rats in our *in vivo* study. Recent evidences indicate, that chronic hyperglycemia may inhibit plasma membrane (PCMA) activity in cells of various tissues. The changes in PCMA can be probably accounted for the hyperglycemia found in our old rats, that in turn may lead to the enzyme protein glycation as shown by Janicki *et al.* (23). In rats with streptozocin-induced diabetes, the brain synaptic PCMA inhibition was accompanied by glycation of hemoglobin. In addition, PCMA activity in synaptic plasma membranes from normoglycemic rats was significantly inhibited by prior incubation with glucose.

In our present *in vivo* study it was only the Mg^{+2}-ATPase, the activity of which has been shown to become gradually higher with age. It has been proved that some functional differences between Na^+, K^+-ATPase and Mg^{+2}-ATPase exist. For example moderate hypoxia increases the activity of synaptosomal Mg^{+2}-ATPase whereas activities of both Na^+, K^+-ATPase and Ca^{+2}-ATPase are found to be decreased in the same conditions (24). Exposure of human synaptosomes to Aß 25-35 (amyloid ß-peptide) resulted in a significant reduction in both Na^+, K^+-ATPase and Ca^{+2}-ATPase activities without affecting Mg^{+2}-dependent ATPase (25). Taken together, our results from *in vivo* study suggest that changes in activity of different P-type pumps may differ with aging and that adaptation of

specific ATPases to internal environment alterations i.e. glucose homeostasis disturbances, is not identical.

We decided to perform *in vitro* study to check if the response of synaptosomal ATPases to glucose in concentrations corresponding to normo- and hyperglycemia *in vivo* is specific and age-dependent. It has appeared that prior incubation with glucose in the concentrations corresponding to normoglycemia in rats *in vivo* (4.5 mM - 6.5 mM) stimulated Ca^{+2}-ATPase activity in the all group of rats, whereas higher glucose concentrations in the incubation medium (10.0 - 12.5 mM) inhibited significantly the enzyme activity. Inhibition of Ca^{+2} - activity was observed in synaptosomes isolated from the oldest rats at relatively low glucose concentration. These data from *in vitro* experiments are consistent with our *in vivo* study, that clearly shows that activity of Ca^{+2}-ATPase in old, hyperglycemic rats was lower than the activity of this enzyme in brain synaptosomes of young normoglycemic animals. Interestingly, as shown by Zaidi and Michaelis, plasma membrane Ca^{+2}-ATPase appears to be very sensitive to the inhibitory effect of reactive oxygen species (ROS) due to the age dependent oxidative modification (26).

By contrast, the activity of Mg^{+2}-ATPase in synaptosomes isolated from brains of old rats was rather low but not inhibited *in vitro*, even at significantly higher glucose concentrations (10.0 - 12.5 mM) corresponding to hyperglycemia *in vivo*. This may explain the increased in vivo activity of Mg^{+2}-ATPase found in old, hyperglycemic rats (with an average blood glucose concentration of 10.26 ± 30 mM). According to Nedeljkovic and co-workers (27) Mg^{+2}-ATPase is not uniformly distributed, differs in respect to affinity for ATP in various rat brain regions and cannot be effectively inhibited by known ATPase inhibitors.

The most sensitive to hyperglycemia *in vitro* seems to be Na^{+}, K^{+}-ATPase in old rats synaptosomes, with the progressive decline starting at 6.5 mM glucose in the incubation medium. This finding is consistent with data of Kaur *et al.* (28), Tanaka and Ando (29), Viani *et al.* (30), Charkraborty *et al.* (31) and Gorini *et al.* (32) indicating decrease of this enzyme activity in brains of aging rats. However, as mentioned before, in our *in vivo* study, Na^{+}, K^{+}-ATPase activity was found only slightly decreased in old rats as compared to the young ones. These differences among the ATPases tested may be accounted for their different function and structure. Na^{+}, K^{+}-ATPase is responsible for maintaining the ionic distribution between intra- and extracellular space being present in almost every cell, including electrically excitable tissues. This protomeric pump consists of α and ß subunits. The subunit α contains the important functional sites of the pump: phosphorylation site, Na^{+} and K^{+}-binding sites and ouabain binding site. The role of heavily glycosylated ß-subunit is not completely clear, however, this subunit is required for the processing, transport and the correct localization of the pump in the plasma membrane. It should be underlined that ß-subunits are not present in the other ATPases (33). Ca^{+2}-ATPase is known to be a regulator of intracellular calcium homeostasis. Two types of Ca^{+2}-ATPase with only marginal homology

have to be distinguished: the sarcoplasmic type and the plasma membrane type (P-type). As for the Na^+, K^+-ATPase the nucleotide-binding and phosphorylation sites are localized on a long intracellular loop of α-subunit. The plasma membrane ATPases differ, however in as much as they possess a calmodulin - binding domain. It seems likely that in the aging brain, multiple methionines within the calmodulin molecule become oxidazed to methionine sulfoxides, resulting in an inability to activate a range of target proteins, including plasma membrane Ca^{+2}-ATPase (34). Mg^{+2}-ATPase, a member of a subfamily of P-type, is presumably responsible for aminophospholipid translocation activity that is necessary for membrane phospholipid asymmetry, observed not only in plasma membranes but also in membranes of other cellular organelles (35).

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