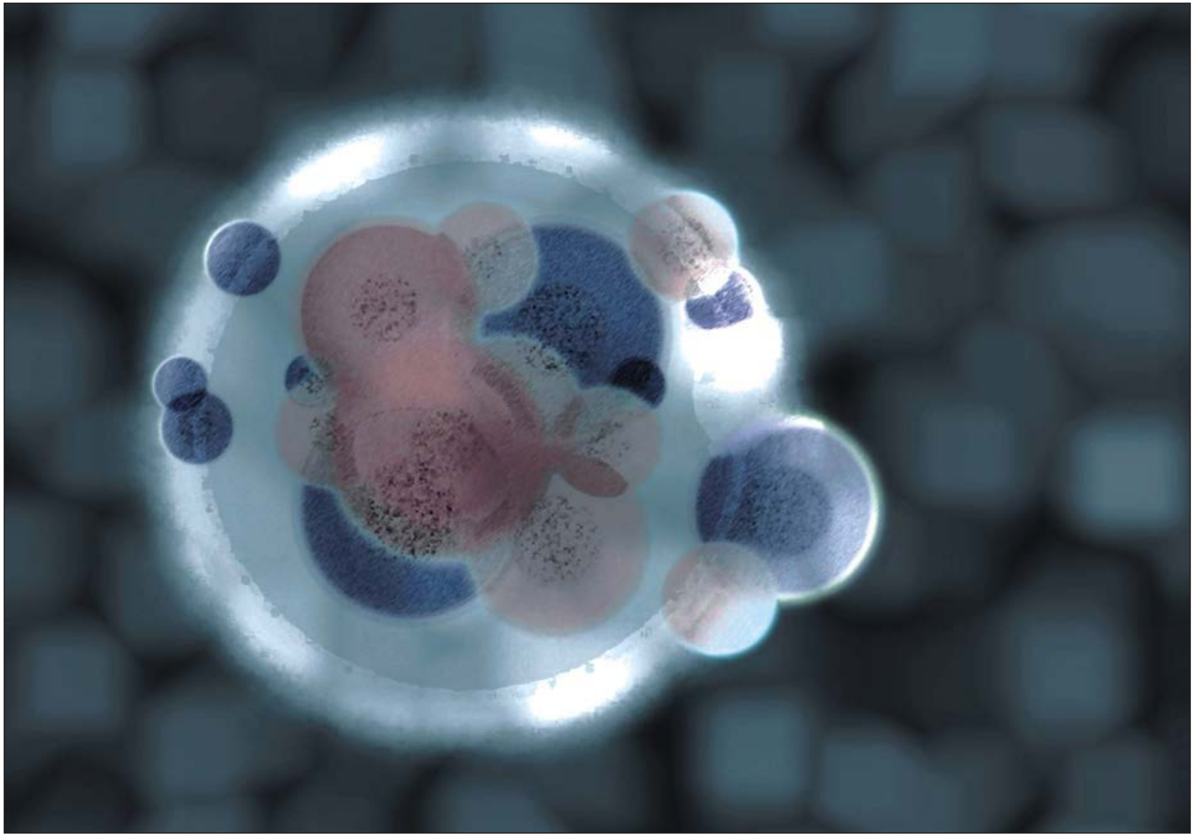

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Effect of Drugs from the Class of Cardiac on the Na^+, K^+-ATPase Activity

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Abstract: The effect of drugs from the class of cardiac (methyldigoxin, verapamil, propranolol), antiepileptic (carbamazepine), sedative (diazepam) and antihistaminic (promethazine) drugs on Na^+, K^+-ATPase activity of plasma membranes in rat brain synaptosomes was studied. Methyldigoxin in concentration of 0.1 mmol L^{-1} inhibits enzyme activity by 80%. Verapamil, propranolol and promethazine in concentrations of 20, 20 and 2 mmol L^{-1}, respectively, inhibit entirely this ATPase activity. Carbamazepine and diazepam in concentrations of 0.02-60 mmol L^{-1} have no effect on the activity of this enzyme. According to drugs concentrations that inhibit 50% of enzyme activity (IC_{50}), the potency order of drugs was: methyldigoxin >> promethazine > verapamil ≥ propranolol. From inhibition of commercially available purified Na^+, K^+-ATPase isolated from porcine cerebral cortex in the presence of chosen drugs, as well as from the kinetic studies on synaptosomal plasma membranes, we may concluded that the drugs inhibit enzyme activity, in partly by acting directly on the enzyme protein. Propranolol, verapamil and promethazine inhibitions were in an uncompetitive manner. The results suggest that these three drugs may contribute to neurological dysfunction and point out to the necessity to take into consideration the side effects of the investigated drugs during the treatment of various pathological conditions.

Key words: Na^+, K^+-ATPase, synaptosomes, verapamil, propranolol, promethazine

INTRODUCTION

Na^+, K^+-ATPase, supporting the ionic homeostasis of the cell, is under control of Na^+, K^+, Mg^{2+} and ATP. Due to significant importance of the Na^+, K^+-ATPase in the maintenance of neuronal resting membrane potentials and propagation of neuronal impulses, the malfunction of this enzyme has been associated with neuronal hyper excitability, cellular depolarization and swelling (Lees, 1991). In numerous tissues, the activities of Na^+, K^+-ATPase may be influenced by different endogenous modulators (Rodrigez *et al*., 1995; Balzan *et al*., 2000; Ewart and Klip, 1995). Na^+, K^+-ATPase activity is decreased by toxic actions of normal neurotransmitters such as glutamate (Brines *et al*., 1995), which is the cause of cell injury and death of neurons, the basic events of cerebral ischemia in epilepsy and in various neurodegenerative disorders (Wyse *et al*., 2000; Grisar, 1984; Lees, 1993). Catecholamines induce marked stimulation of Na^+, K^+-ATPase activity by stimulation of β2-adrenoceptors, leading to hyperpolarization of the cell membrane (Clausen and Flatman, 1977).

With regard to the importance of this enzyme for the proper functioning of cells and tissues and in the induction of cytotoxicity, especially in nerve cells, the present study was undertaken in order to examine the effects of particular drugs on Na^+, K^+-ATPase. The effect of drugs from the class of cardiac (methyldigoxin, verapamil, propranolol), antiepileptic (carbamazepine), sedative (diazepam) and antihistaminic (promethazine) drugs on Na^+, K^+-ATPase in synaptic plasma membranes prepared from the whole rat brain were investigated. Known pharmacological effects of these drugs are not detected concerning the synaptosomal Na^+, K^+-ATPase activity. Methyldigoxin was known to inhibit Na^+, K^+-ATPase in various tissues but it was included in our experiments with the purpose to compare its efects with effects of other two anthyarrhitmic drugs. For propranolol, there is litle evidence while for verapamil and promethasine there is no information of their effects on brain Na^+, K^+-ATPase. Carbamazepine and diazepam were examined because their effect on synaptosomal Ca-ATPase, sodium channels and ATPase were priviously observed, but there is no data of their Na^+, K^+-ATPase activity modulation. Also, the effects of the chosen dugs on the commercial Na^+, K^+-ATPase from porcine brain were examined with the aim to evaluate if the effects of these drugs may be directly on the enzyme protein. In addition, extensive kinetic studies were undertaken to determine the nature of the drugs action.

MATERIALS AND METHODS

Chemicals: Methyldigoxin (β-Methyldigitoxin) was obtained from ICN Pharmaceuticals, Inc., USA. Adenosine 5`-triphosphatase (sodium- and potassium-activated, ouabain sensitive and vanadate inhibited; EC 3.6.1.3.) purified from porcine cerebral cortex (ouabain sensitive activity: 0.4 U mg^{-1} protein), propranolol chloride (1-[isopropylamino]-3-[1-naphthyloxy]-2-propanol), verapamil chloride, carbamazepine (5H-Dibenz[b, f]azepine-5-carboxamide), diazepam (7-Chloro-1-methyl-5-phenyl-3H-1,4 benzodiazepine-2(1H)-one) and promethazine and all other chemicals were purchased from Sigma-Aldrich (Germany).

Synaptosomal plasma membrane preparation: Experiments were performed on 3-month-old male Wistar albino rats obtained from the local colony. Animals were kept under controlled illumination (lights on: 7:00-19:00 h) and temperature (23±2°C) and had free access to commercial rat pellets and water. All experiments with animals were performed in accordance to the current European Convention. After decapitation with a small animal guillotine, the brains from 6 animals were rapidly excised for immediate Synaptosomal Plasma Membranes (SPM) isolation. The SPMs were prepared according to the method of Towle and Sze (1983). The preparation procedure of SPM preparation was described previously (Horvat *et al*., 1995). The purity of membrane preparation was analyzed in our preview experiments by electron microscopy and by activity of membrane specific enzyme. From micrography of SPM we observed that the preparation consists mainly of membrane vesicles, without significant contamination by other organelles including mitochondria, nuclei, lysosomes or endoplasmatic reticulum (Peković, 1986). Inhibition of ATP hydrolysis by specific inhibitor of Na^+, K^+-ATPase, ouabain about 80%, as well as the presence of adenylate cyclase activity also confirmed high levels of SPM in our preparation (Peković *et al*., xtracellular side of plasma membrane (Forbush, 1982; Kinne-Saffran and Kinne, 2001). In our preparation of SPM, about 55% of ATPase activity detected was accessible to ouabain, indicating to a proportion of right-side of SPMs which were exposed to glycoside representing non-sealed, broken SPM vesicles or leaky vesicles (right-side out vesicles exposes ouabain site but the activity of Na^+, K^+-ATPase coud not be detected since ATP sites are inside of vesicles) (Forbush, 1982; Lopez *et al*., 2002). To determine vesicle orientation we also applied Sodium Dodecyl Sulfate (SDS) and Tween 20 (Tw20) to permeabilize or open all vesicles. Since in right-side vesicles ATP site of Na^+, K^+-ATPase is unaccessible, the increment in activity in SDS or Tw20 treated and untreated would be expected to result from accessibility gained by the substrate to its binding site of the ATPase. According to Na^+, K^+-ATPase activity detected in the presence of 0.2 mg of SDS mg^{-1} SPM proteins or 2% Tw20 for 20 min before enzyme assay, which was increased about 2-fold (0.518 μmol Pi mg^{-1} min^{-1} for SDS and Tw20) in respect to non SDS/Tw20 treated (0.249 μmol Pi mg^{-1} min^{-1}), we concluded that 52% of total Na^+, K^+-ATPase activity are exposed by SDS and that their values represent a proportion of activity existing in right-side out sealed vesicles (Gill *et al*., 1986). This proportion of inverted and leaky vesicles and membrane fragments was 48%. The results obtained from both treatments indicate that our membrane preparation mainly consists of right-side out vesicles (52%), leaky vesicles and non- sealed membrane fragments (26%) and lower proportion (22%) of inside-out oriented vesicles.

RESULTS

Effects of drugs on Na^+, K^+-ATPase activity: Effects of cardiac, antiepileptic, sedative and antiallergic drugs on the ATP hydrolytic enzyme activity were examined in isolated synaptic plasma membranes from whole rat brains. *In vitro* incubation of SPM with MDO, PPNL, VP and PMZ for 30 min produce a dose-dependent inhibition of Na^+, K^+-ATPase activity. Anticonvulsant and sedative drugs, CMZ and DZ do not affect the activity of brain Na^+, K^+-ATPase (data not shown).

Figure 1 represents the dose-dependent inhibition of Na^+, K^+-ATPase by drugs. It is known that digoxin inhibits cardiac Na^+, K^+-ATPase interacting with the ouabain-binding site on the α catalytic subunit of the enzyme (Jortani and Valders, 1997; Lingrel *et al*., 1998; Repke *et al*., 1995).

Comparing the effects of ouabain and MDO (data not shown), we concluded that brain Na^+, K^+-ATPase possessed a higher affinity for ouabain, but these two drugs produced similar shaped inhibition-curves (IC_{50} were 2.195±0.42 μmol L^{-1} for ouabain and 2.97±0.38 μmol L^{-1} for MDO).

Higher affinity of ouabain binding sites to ouabain than for digoxin were also found in ox and rat brain frontal cortex membranes (Mazzoni *et al*., 1990; Acuna Castroviejo *et al*., 1992). Maximum inhibition of the enzyme was achieved in the presence of 0.1 mmol of MDO. Incubation of SPM with antiarrhytmic drugs PPNL

Table 1: IC_{50}, percentage of maximum inhibition and Hill coefficient (n) from *in vitro* application of various drugs on SPM (20 μg) and commercial porcine brain cortex (0.0078 U) Na^+, K^+-ATPase activity

	IC_{50}(mmol L^{-1})		Inhibition (%)	
Drug	SPM	Commercial	SPM	Commercial
Methyldigoxin	0.00297±0.00038		80	
N	0.697±0.066			
Propranolol	3.07±0.24	3.15±0.04	94	95
N	1.13±0.14	1.51±0.19		
Verapamil	1.9±0.14	2.01±0.51	98	95
N	1.41±0.05	1.86±0.03		
Promethazine	0.84±0.0005	0.165±0.02	98	95
N	2.62±0.29	1.80±0.23		
Diazepam	no		no	
Carbamazepine	no		no	

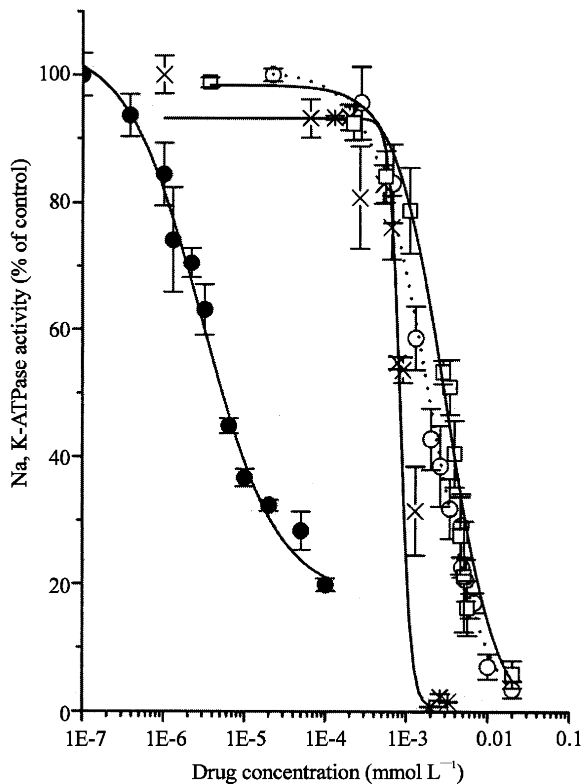

Fig. 1: Inhibition curves of Na^+, K^+-ATPase from SPM in the presence of various drugs. SPM (20 μg of proteins) were incubated 30 min in the presence of 0.1-100 μmol L^{-1} of methyldigoxin (●), 0.001-20 mmol L^{-1} of propranolol (◘), verapamil (○) or promethazine (x) without ATP. After incubation, 2 mmol L^{-1} ATP (Tris-salt) was added and the enzyme reaction lasted 15 min. Results represent Mean percentage of enzyme activity in respect to control velocity, without drugs (0.420 μmol Pi mg^{-1} min^{-1}) ±SEM, as determined from five separate experiments, each assayed in triplicate

and VP similarly inhibited Na^+, K^+-ATPase activity in a dose- dependent manner. The inhibition was significant to the $p = 0.002$ level at concentrations greater than 0.5 mmol L^{-1} for both drugs. Maximum enzyme activity inhibition of PPNL and VP were achieved at concentrations of 20 mmol L^{-1}.

The antihistaminic drug, PMZ, exerts a higher inhibition effect than the former two antiarrhytmic drugs with maximum inhibition at the concentration of 2 mmol L^{-1}. Significant enzyme activity inhibition was also detected at concentrations greater than 0.1 mmol L^{-1}.

Dixon plots (Dixon and Web, 1987) of data for all drugs applied were used to determine whether drug binding was in equilibrium with inhibitory sites on the enzyme by plotting 100/(100-% inhibition) vs. drug concentration. Linear Dixon plots implying equilibrium binding were obtained in all cases. The half-maximum inhibition (IC_{50}) was calculated from the Hill analysis of the experimental results. The IC_{50} values and Hill coefficient, n, determined from inhibition curves by the Hill analysis are summarised in Table 1. According to IC_{50} values for applied drugs, the potency order of drugs was: methyldigoxin >> promethazine > verapamil ≥ propranolol.

To evaluate if these drugs exert their effects acting directly on the sodium pump, we incubated Na^+, K^+-ATPase isolated and purified from porcine cerebral cortex (commercially available) and from SPM preparations (Fig. 2). The effect of MDO was not examined since it is known that this drug binds to the ouabain site on α subunits of the enzyme, as mentioned above. PPNL and VP identically inhibited enzyme activity from both sources (Fig. 2a,b). PMZ exerted total inhibition of Na^+, K^+-ATPase activity from both sources (Fig. 2c), according to IC_{50}, commercial Na^+, K^+-ATPase preparation possessed about 4 fold higher sensitivity than in the SPM preparation. Table 1 shows data obtained with *in vitro* incubation of SPM and commercial Na, K-ATPase.

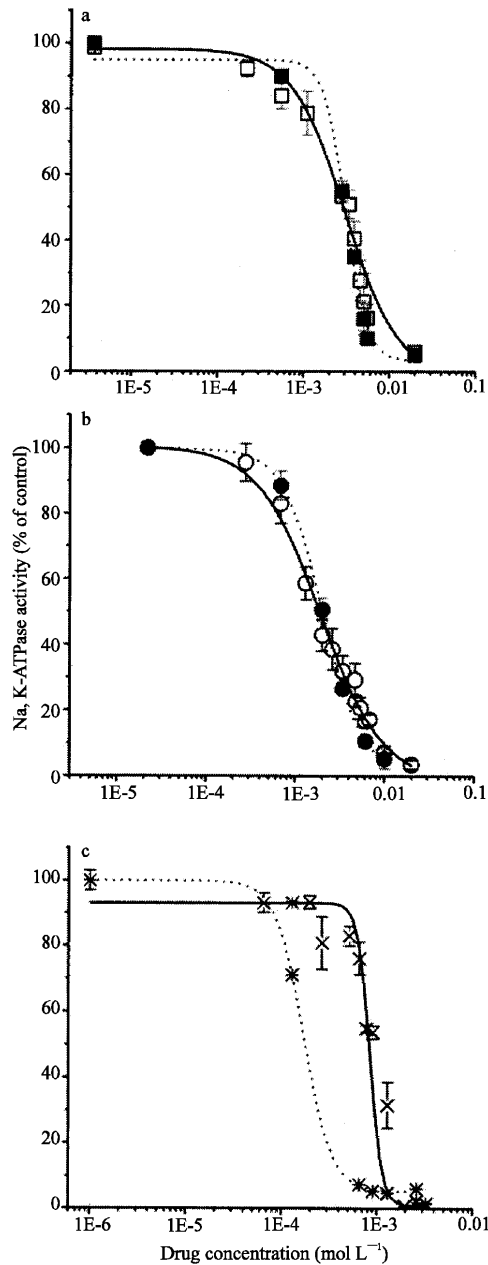

Fig. 2: Inhibition of Na^+, K^+-ATPase activity from SPM and purified-commercial enzyme. SPM (20 μg) and commercial Na^+, K^+-ATPase isolated from pig brain cortex (0.0078 U) were incubated with: a) propranolol (◘ for SPM, ■ for commercial enzyme), b) verapamil (○ for SPM, ● for commercial enzyme), or c) promethazine (x for SPM, * for commercial enzyme). Incubations of both enzymes were done as denoted in the legend of Fig. 1. The results represent Mean percentage of enzyme activity in the presence of drugs in respect to control velocity±SEM, as determined from five separate experiments, each assayed in triplicate

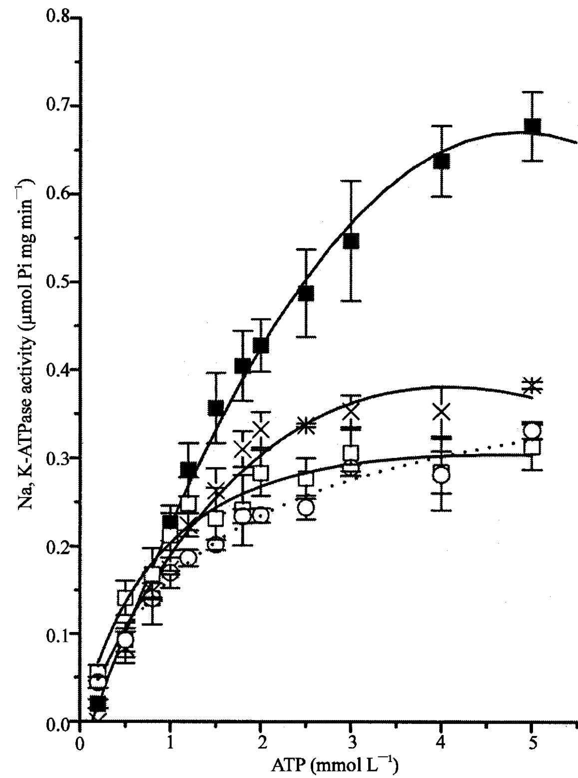

Fig. 3: Concentration-dependent activation of Na^+, K^+-ATPase with ATP. SPM (20 μg) was incubated without (■) and with 1 mmol L^{-1} of promethazine (x), 2 mmol L^{-1} of verapamil (○-dot line) or 3 mmol L^{-1} of propranolol (◘) for 30 min. After incubation, the enzyme assay was started by the addition of increasing concentrations of ATP (0.2-5 mmol L^{-1}). Results are presented as μmol Pi mg^{-1} min^{-1}±SEM, as determined from five separate experiments, each assayed in triplicate

Mechanism of action: To evaluate the nature of enzyme inhibition, the kinetic analyses of the effects of drugs mentioned above on the enzyme activation by substrate were carried out. Kinetic parameters, V_{max} and K_m, were determined by varying the concentration of ATP (0.2-5 mmol L^{-1}) in the presence and absence of the mentioned drugs. The effect of MDG on the kinetic properties of Na^+, K^+-ATPase was not examined for the reason mentioned previously. The effects of PPNL, VP and PMZ were determined in the presence of concentrations of 3, 2 and 1 mmol L^{-1}, respectively. These particular concentrations were chosen from the inhibition curves, as the IC_{50} concentrations.

The dependence of the reaction rate vs. ATP concentration for Na^+, K^+-ATPase in the presence and absence of chosen drugs exhibited typical Michaelis-Menten kinetics (Fig. 3). Kinetic constants, K_m and V_{max},

Table 2: Kinetic parameters of Na^+, K^+-ATPase from SPM in the absence (control) and presence of 3, 2 or 1 mmol L^{-1} propranolol, verapamil or promethazine, respectively in incubation mixture (20 μg of SPM proteins)

	K_m mmol L^{-1}	V_{max} μmol Pi min^{-1} mg^{-1}	Type of inhibition	Hill coefficient (n) for ATP
control	3.589±0.460	1.188±0.120		0.95±0.07
propranolol	1.272±0.215	0.346±0.023	Uncompetitive	1.59±0.19
verapamil	1.409±0.163	0.388±0.019	Uncompetitive	1.40±0.12
promethazine	1.079±0.368	0.470±0.058	Uncompetitive	1.03±0.08

were calculated from the Eadie-Hofstee transformation of experimental data and are shown in Table 2. The type of inhibition were analysed from double-reciprocal plots of velocity vs ATP concentrations. However, Km as well as Vmax values decreased in the presence of all three drugs indicating an uncompetitive type of inhibition.

DISCUSSION

In this study we have investigated the effects of various drugs, whose known pharmacological effects were not on the rat brain synaptosomal Na^+, K^+-ATPase activity. The present study shows that antiarrhytmic drugs propranolol (β-adrenergic receptor antagonist) and verapamil (calcium channel blocker) as well as antihistaminic drug promethazine (histaminic H1 receptor blocker) inhibit rat brain Na^+, K^+-ATPase activity in a dose-dependent manner. In contrast, tricyclic antidepressant-carbamazepine and anticonvulsant, diazepam had no effects on Na^+, K^+-ATPase activity.

The results on cardiac sarcolemma showed that various antiarrhytmic drugs, other than PPNL and VP, inhibit Na^+, K^+-ATPase activity by interacting with the same or similar receptor site as ouabain (Almotrefi *et al*., 1999). To compare the efficiency of cardiac drugs, PPNL and VP, as a control effect, we measured the inhibition of the enzyme activity in the presence of cardiac glycoside, ouabain and MDO. It is well known that digoxin is a very potent and specific inhibitor of Na^+, K^+-ATPase activity in heart cells (Almotrefi *et al*., 1999) and *in vivo* of kidney and red cells (Rodriguez *et al*., 1994). Also, inhibitory effects of *in vivo* applied digoxin have bee seen on Na^+, K^+-ATPase in the liver, muscle, renal medulla and aorta (Li *et al*., 1993). The catalytic subunit of the enzyme is a site of inhibitory action of cardiac glycoside. In the rat brain, at least three isoforms of the α (α1, α2 and α3) subunit have been predicted from cDNA cloning experiments (Sweadner, 1985; Herrera *et al*., 1987; Jewell *et al*., 1992) and they differ in amino acid composition, molecular weight, sensitivity to ions and cardiac glycoside like ouabain. Three ouabain inhibitory sites were detected and named: low-, high- and very high-affinity with IC_{50} in the range of m-, μ- and nmol L^{-1}, respectively. These sites contribute to three α subunit isoforms (α1 21%, α2 50% and α3 17%) of Na^+, K^+-ATPase (Peković *et al*., 1997; Berrebi-Bertrand *et al*., 1990). Inhibition of SPM Na^+, K^+-ATPase activity by ouabain and MDO we tested, were similar as expected. Since their concentrations were in the range of μmol L^{-1}, it means that these inhibitors occupy very high- and high-affinity binding sites (α2 and α3) on Na^+, K^+-ATPase. Percentages of inhibition of 80% by MDO indicate to an abundance of these α isoforms in the SPM preparations as we found out for ouabain in our earlier investigation (Peković *et al*., 1997). Higher IC_{50} for MDO we found, which is in agreement with ouabain and MDO inhibition of [^{3}H]ouabain binding to rat cerebral cortex SPM and ox frontal cortex membrane (Acuna Castroviejo *et al*., 1992; Mazzoni *et al*., 1990) may be explained by higher affinity of ouabain binding sites for ouabain. The β-adrenoceptor antagonist, PPNL and VP, Ca-channel blocker, are less potent antiarrhythmic drugs in respect to MDO with higher IC_{50} values. These findings may indicate that the acting site of PPNL and VP is not the ouabain site on the enzyme. PPNL and VP inhibit 94- 98% of Na^+, K^+-ATPase activity and according to IC_{50}, VP was the slightly more potent drug in respect to PPNL. It was found that PPNL, above concentrations of 20 μmol L^{-1}, affects membrane enzyme activity in cardiac sarcolemmal membranes by increasing membrane fluidity (Clatelain *et al*., 1989). Membrane fluidization with PPNL has also been observed on rat platelet membranes (Nosal *et al*., 1985) rat erythrocyte membranes (Weitman *et al*., 1989) and rat brain membranes at concentrations higher than 10 mmol L^{-1} (Ondrias *et al*., 1987; 1989). Using electron spin resonance techniques, it was found that PPNL fluidizes these membranes in a depth-specific fashion by changing fluidity in the hydrophobic membrane thus influencing lipid-protein interaction. On the contrary, in cultured human and murine fibroblasts, it was found that PPNL exerts no effect on membrane fluidity (Eggl *et al*., 1986) and composition of phospholipids (Schroeder *et al*., 1981). The discrepancy in PPNL effects on membrane fluidity may be a consequence of tissue specific characteristics in the membrane content of some phospholipide species and cholesterol.

As for PPNL, data from the literature indicate that VP possesses a perturbation effect on the lipid part of liposomal membrane prepared from rat platelets

(Ondriasova *et al*., 1992) and on rat brain total lipid liposomes (Ondrias *et al*., 1991, 1992). VP possesses a bulk hydrophobic mass and its partition into lipid matrix was found to decrease carrier-mediated ion flux at a concentration > 10 mmol L^{-1} (Shi and Tien, 1986). In our examination, total inhibition of Na^+, K^+-ATPase with VP was found at a concentration < 10 mmol L^{-1}. Also, the simmilarities we have seen in IC_{50} for PPNL and VP on Na^+, K^+-ATPase and on ecto-ATPase of rat SPM (unpublished data), which activity is insensitive to fluidity parmeter (Bloj *et al*., 1973), indicate that the effects of these drug is not only by memmbrane fluidization. Inhibition by VP and its metabolites at concentrations greater than 0.1 mmol L^{-1} of NTPDase in vascular endothelial cells were seen and Ki of drugs ranging from 0.6-3.9 mmol L^{-1} (Gendron *et al*., 2000). Our results with purified Na^+, K^+-ATPase indicate similar effects of the investigated drugs on the enzyme affinity and velocity. Since we found that PPNL and VP inhibited SPM Na^+, K^+-ATPase activity in the same extent as purified Na, K-ATPase, it may be proposed that the drugs exert their effects by direct action on the brain enzyme protein and not by disturbing membrane fluidity. We have however no information of lipid content in the commercially available enzyme. Since for the activity of Na^+, K^+-ATPase, a phospholipid environment is necessary, it may be postulated that the purified Na^+, K^+-ATPase consists of the enzyme incorporated in phospholipids. PPNL and VP by inserting in the hydrophobic part of membrane phospholipids in the nearest portion of membrane to catalitic suburnits may affect the conformational change of enzyme protein, but the effects on the extramembrane part of ATPase may not be excluded.

As confirmation of existence of extramembrane site of action of anthiarrithmic drugs was the calculated Hill coefficient from inhibition data, indicating positive cooperativity in VP action, no cooperativity in PPNL action and negative cooperativity in MDO action. Since the type of inhibition of these cardiac drugs were different, we may conclude that they acted on different sites on the enzyme. According to kinetic parameters, the inhibitions of PPNL and VP were uncompetitive, decreasing maximum velocity and affinity for ATP. Both drugs change the type of ATP binding to Na^+, K^+-ATPase, from noncooperative to positive cooperativity type in respect to the enzyme under control conditions.

In present study ATP hydrolising activity of the enzyme may be detected in 48% of isolated SPM, the high drug/protein concentrations ratio was a consequence of high percentage of right-side out vesicles which binds the drugs and thus the effects could not be detected. According to this drug/protein ratio it is half of that found in present study.

It was shown that some beta-blocking agents inhibit Na^+, K^+-ATPase in cultured corneal endothelial and epithelial cells up to 78% (Whikehart *et al*., 1991). The results of Gopalaswamy *et al*. (1997) showed that PPNL inhibited brain Na^+, K^+-ATPase, Mg^{2+}-ATPase and Ca^{2+}-ATPase activity. These authors found that IC_{50} for all three enzymes were 1.5-1.8 mmol L^{-1} and that inhibition of Na^+, K^+-ATPase was of an uncompetitive type with respect to ATP. Our results were similar to those previously reported in respect to Na^+, K^+-ATPase, minor discrepancy in the results may be caused by differences in the enzyme assay. We included in this study PPNL with the aim to determine its IC_{50} order in respect to other drugs we have studied.

Up to now, according to our knowledge, the effect of VP on brain Na^+, K^+-ATPase was not studied. It was shown that some calcium channel blocking agents inhibit the activity of Na^+, K^+-ATPase in myocardial sarcolemma (Dzuba *et al*., 1991) and for VP specific inhibition of the calmodulin-stimulated (Ca^{2+} + Mg^{2+})-ATPase activity in human erythrocyte, rat and guinea pig ventricular sarcolemma and rat brain synaptosomes (Raess and Gerstten, 1987; Kim and Raess, 1988; Raess and Record, 1990; Hoechen, 1977; Dong and Xue, 1994).

The effect of PMZ on brain Na^+, K^+-ATPase was not examined up to now. It was found that some antiallergic drugs with anti-inflammatory action, but not PMZ, may increase the depressed platelet levels of Na^+, K^+-ATPase activity, observed in allergic subjects and it was proposed that this modulation of the enzyme activity could be a possible mechanism of action for these drugs (Gentile *et al*., 1993). Some phenothiazines accumulate in the brain provoking dopamine receptor blockade (Paglini-Oliva and Rivarola, 2003) and in rat hepatocytes, promethazine possesses an antioxidant effect (Albano *et al*., 1991). In human erythrocytes, it was proposed that cationic drug, PMZ bound to and/or penetrated the intermembranes and induced haemolysis as a result of drug binding, forming mixed micelles with the membrane and disrupting membrane structure (Yamamoto and Aki, 1991). Inhibition of Na^+, K^+-ATPase activity by PMZ has been seen in filarial parasite Setaria cervi (Agarval *et al*., 1990). According to our results presented in this study, PMZ is a potent inhibitor of rat brain SPM Na^+, K^+-ATPase activity producing total enzyme inhibition at concentrations of 2 mmol L^{-1} and with IC_{50} of 0.84 mmol L^{-1}. Commercial, pure porcine brain Na^+, K^+-ATPase was more sensitive to PMZ and IC_{50} was calculated to be 0.165 mmol L^{-1}. The lower efficiency of PMZ on SPM Na^+, K^+-ATPase activity may be due to the environmental milieu in plasma membrane and partial coverage of binding site for PMZ, which is

uncovered in the commercial preparation of the enzyme and by sidedness of SPM vesicles. Another possibility is the different species-dependent sensitivity of synaptosomal Na^+, K^+-ATPase. The effect on the pure commercial enzyme indicates that this drug directly affects the enzyme but these effects did not exclude effects via damage of the membrane, alteration in membrane fluidity and damage of cytoskeleton. Although the effect described in this paper, on ATP dependent Na^+, K^+-ATPase activity indicates that this drug affects enzyme activity by decreasing both velocity and affinity for ATP (60 and 70%, respectively) which indicates to uncompetitive inhibition. The Hill coefficient calculated from inhibition data, n>1 indicates to the existence of positive cooperativity in the enzyme activity inhibition. PMZ in contrast to PPNL and VP did not change the no-cooperativity type of ATP binding to the enzyme (n = 1).

It was found that the anaesthetic drug, pentobarbital in concentration of 50 μmol L^{-1}, decreases two-fold the percentage of phosphatidylinositol, phosphatidylglicerol and phosphatidylserine which is intimately involved in divalent ion binding to membranes and may partially regulate activities of membrabe-bound and ion transporting enzymes (Schroeder *et al*., 1981). Exploring pentobarbital action on ouabain receptor affinities of three isoforms of the catalytic subunit of Na^+, K^+-ATPase in rat brain and Na dependance of the enzyme activity, it was found that pentobarbital-induced anaesthesia, induced fatty acid modification of brain membranes and significant sensibilization to ouabain of α2 and α3 isoforms. The authors concluded that pentobarbital altered properties could be related to a selective modification of the fatty acid composition and/or to the presence of a specific binding site for pentobarbital on these two neuronal digitalis receptors (Gerbi *et al*., 1997). Ondrias *et al.* (1983) found that local anaesthetics may incorporate into the lipid part of synaptosomes from rat brain and induce perturbation of the membrane. In our experiments, tricyclic antidepresant CBZ and anticonvulsant DZ which are anaesthetics, had no effect on Na^+, K^+-ATPase activity in synaptosomal membranes which could be expected if they exert perturbance of the membrane lipid bilayer. The omission of the effect of CBZ and DZ indicates that these drugs did not induce membrane lipid perturbation.

Abnormal functioning of synaptosomal Na^+, K^+-ATPase may be the cause of many different types of neurological disorders, since constant depolarizations of the cell membrane induce abnormally excessive amounts of certain neurotransmitters to be released. As a consequence of reductions in sodium pump function, by the drugs we have explored, destruction of the sodium gradient which drives the uptake of acidic amino acids and a number of other neurotransmitters may occur. This results in both a block of reuptake and a stimulation of the release not only of glutamate but also of other neurotransmitters which modulate the neurotoxicity of glutamate. An exocytotic release of glutamate and other neurotransmitters can also occur because of depolarization of the membrane as a consequence of inhibition of the enzyme (Lees, 1991). Also, increased intracellular concentrations of Na^+ as a consequence of Na^+, K^+-ATPase inhibition, will increase its exchange for Ca^{2+} by Na/Ca exchanger which may induce extensive neurotransmission. Retention of sodium may result in osmotic swelling and possible cellular lysis.

It was shown that the investigated drugs *in vivo* may pass the blood brain barrier and may even accumulate in the brain of rodents and humans nerve endings and released by membrane depolarization (Myers *et al*., 1975; Srivastava and Katyare, 1983; Street *et al*., 1984; Bright *et al*., 1985; Mariyama *et al*., 1993; Hendrikse *et al*., 1998). Thus, our findings on the inhibition of brain Na^+, K^+-ATPase activity by the selected drugs *in vitro*, may be related to *in vivo* effects of these drugs. Under various pathological states, decreased activities of Na^+, K^+-ATPase were found as well as in the aged brain (Murakami and Furvi, 1994; Park, 1994). In the human actively spiking regions of epileptic temporal cortex, decreased synaptosomal Na^+, K^+-ATPase activities were found (Nagy *et al*., 1990; Nagy, 1997). Additional inhibition of this enzyme with drugs, investigated in this work, may lead to further synaptosomal hyperactivity and increased brain tissue excitability in the epileptic brain. In conclusion, our findings point out to the necessity of considering the side effects of the investigated drugs when treating various pathological conditions.

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