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PHENYTOIN DEPHOSPHORYLATES THE ALPHA(−) CATALYTIC SUBUNIT OF (Na^+, K^+)-ATPase

A STUDY IN MOUSE, CAT AND HUMAN BRAIN

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Abstract—Phenytoin, a potent antiepileptic drug, has been thought to stimulate Na^+, K^+ transport across cell membranes, but its influence on (Na^+, K^+)-ATPase activity remains highly controversial. We have investigated the effects of the drug on the phosphorylation level of (Na^+, K^+)-ATPase partially purified from mouse, cat and human brain. (Na^+, K^+)-ATPase catalytic subunits [alpha(+) and alpha(−)] were resolved by sodium dodecylsulfate polyacrylamide gel electrophoresis. Previous experiments had shown that phenytoin dephosphorylates the (Na^+, K^+)-ATPase catalytic subunit by ±50% in C57/BL mice. In the present study, we showed that phenytoin (10^{-4} M) decreases the phosphorylation level of (Na^+, K^+)-ATPase catalytic subunit by the same value in cat and human cortex. Moreover, that effect is predominant on the alpha(−) subunit, thought to be the predominant enzymatic form in non-neuronal or glial cells. The results are thus favoring the hypothesis that phenytoin stimulates the brain (Na^+, K^+)-ATPase. They further suggest that phenytoin mainly activates the glial enzymatic form, providing central nervous system with an enhanced ability to regulate extracellular K^+.

(Na^+, K^+)-ATPase¶ (ATP phosphohydrolase; EC 3.6.1.3) is an ubiquitous membrane bound protein, catalysing the active transport of Na^+ and K^+ ions across animal cell membranes. Present in high concentration in the brain, it has the important role of maintaining the ionic gradients necessary for neuronal excitability [1, 2]. Sodium dodecylsulfate gel electrophoretic studies of purified (Na^+, K^+)-ATPase show mainly two polypeptides: a large subunit (called alpha; MW 85,000–130,000), referred to as the catalytic subunit, and a small glycosylated subunit (called beta; MW 40,000–60,000), whose function is still unknown [3, 4]. More recently, three isoenzymes of the (Na^+, K^+)-ATPase catalytic subunit have been identified, two as proteins (alpha(−) and alpha(+) on SDS-gel electrophoresis) [4], and a third as a cDNA and mRNA [5]. The two molecular forms alpha(−) and alpha(+) have different sensitivities to ouabain inhibition [4], to pyrithiamin inhibition [6] and to K^+ dephosphorylation [7], different antigenic determinants [8] and different amino acid sequences [9].

In rat brain, each of the two enzymes was initially assigned to a different cell type: alpha(−) to non neuronal or glial cells and alpha(+) to axolemma. Some observations have since complicated that simple model and are consistent with the view that several forms of (Na^+, K^+)-ATPase may be present in the same brain cell type [5, 10]. However, alpha(−), the kidney type catalytic subunit, appears to be predominant in non neuronal, glial cells [4].

Phenytoin has been suggested to work as an antiepileptic drug by stimulating the [Na^+, K^+] pump and the (Na^+, K^+)-ATPase. Woodbury first demonstrated that PHT increased Na^+ turnover in whole brain, where it prevented the rise in intracellular Na^+ level caused by maximal electroshock seizures [11]. These experiments were the basis for Woodbury's hypothesis that the anticonvulsant effects of PHT could result from its enhancement of active Na^+, K^+ transport across cell membranes. This concept found support in subsequent studies [12, 13], which showed that PHT stimulates (Na^+, K^+)-ATPase activity and synaptic potassium uptake in ionic conditions simulating a depolarized state. More recently, it has been shown that therapeutic concentrations of PHT increased permeability of cultured glial cells to K^+, resulting in hyperpolarization [14]. However, studies on the direct effects of PHT on (Na^+, K^+)-ATPase specific activity have shown conflicting results [15]: no effects, stimulation or even inhibition of the enzyme. Other authors have thus proposed that PHT could inhibit (Na^+, K^+)-ATPase and block the passive inward movements of Na^+ [16].

The following work was undertaken to assess directly the effects of PHT on partially purified (Na^+, K^+)-ATPase from mouse, cat and human brain. Our experiments demonstrate that PHT dephospho-

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¶ Abbreviations used: (Na^+, K^+)-ATPase; sodium and potassium ion-stimulated adenosine triphosphatase; PHT, phenytoin; SDS, sodium dodecylsulfate; MW, molecular weight; Kd, kilodalton.

Table 1. Wet weights, protein microsomal contents and ATPases activities of C57/BL mice, cats and humans cortex

	C57/BL mice	Cats	Humans
Wet weight (g)	1.07 ± 0.06	0.71 ± 0.07	0.66 ± 0.36
Protein microsomal content (mg/g of wet wt)	2.22 ± 0.22	4.33 ± 2.14	5.37 ± 1.61
Total ATPase	231 ± 35	156 ± 22	95 ± 36
Mg^{2+}-ATPase	113 ± 17	70 ± 10	35 ± 16
(Na^+, K^+)-ATPase	118 ± 19	84 ± 14	60 ± 24

ATPase activities are expressed in μmol P_i/mg protein/hr and correspond to the fraction with maximal (Na^+, K^+)-ATPase activity obtained after centrifugation on the sucrose gradient. Mg^{2+}-ATPase activity is measured in presence of 1 mM ouabain. (Na^+, K^+)-ATPase activity is calculated by the difference between total ATPase and Mg^{2+}-ATPase activities. Results are mean of 9 (mice), 3 (cats) or 4 (human) determinations ± SD values.

rylates the catalytic subunit of (Na^+, K^+)-ATPase from normal brain and acts predominantly on its alpha(−)subspecies.

MATERIALS AND METHODS

Tissue origin. C57/BL mice (21 days old) were purchased from the Jackson Laboratories. Brains were removed immediately after decapitation of the mice and processed without delay. White matter, brainstem, and basal ganglia were dissected out and eliminated. Each experiment pooled samples from four animal brains.

Adult cats (1.5–4 kg) of either sexes were obtained from the University Vivarium. Anesthesia was induced by halothane inhalation given by facial mask. After subcutaneous injection of atropine (0.05 mg/kg), endotracheal intubation or tracheotomy was performed in order to carry on anesthesia under halothane/O_2. Cats were then placed into a stereotaxic frame; a large craniotomy was performed and dura was opened, exposing the cortical convexity of both hemispheres. Wound edges and pressure points were infiltrated with subcutaneous 1% xylocaine and gallamine triiodoethylate (2 mg/kg) was given intravenously. Respiration was assisted artificially and adjusted in order to maintain the venous pH between 7.3 and 7.5. Electrocorticography (ECOG) was then monitored by silver electrodes on both hemispheres connected to an electroencephalograph (Siemens Mingograph 8). During electrical activity recordings, halothane anesthesia was discontinued. After obtaining a stable activity for 2 to 3 hr typical of the awake cat, cerebral cortex was excised in the right suprasylvian gyrus. White matter was carefully dissected out and eliminated.

Control human cortex was obtained from a patient who died because of a myocardial infarction (patient 1). Three patients were operated on for a deep brain lesion: hematoma (patient 2) or tumor (patients 3 and 4). Those 3 patients never suffered from clinical seizures and repeated EEG's were consistent with the presence of a lesion, without signs of paroxysmal activities. Macroscopically normal frontal cortex was excised in order to gain access to the deep lesion (frontal glioma and astrocytoma). White matter was eliminated.

Tissue processing. The cerebral cortex (0.3–1.1 g, Table 1) was homogenized on ice in 10 vol. of 0.32 M sucrose, 1 mM EDTA, and 25 mM imidazole, pH 7.4, with a motor-driven Teflon-glass homogenizer. The homogenate was centrifuged for 20 min at 850 *g* to remove nuclei and unhomogenized material. The supernatant was centrifuged for 20 min at 8500 *g*. The pellet (mitochondria and myelin fragments) was discarded, and the supernatant was centrifuged for 1 hr at 130,000 *g*. The pellet, which consisted of the microsomal fraction, was resuspended in the above sucrose/EDTA/imidazole buffer, pH 7.4, to a final protein concentration of 4 mg/ml.

Partial purification of (Na^+, K^+)-*ATPase.* The microsomal fraction was incubated at room temperature for 30 min with 2 mM EDTA, 3 mM imidazole, pH 7.4, and with SDS (with a maximum of 0.3 mg of SDS/ml). After incubation, SDS-treated microsomes (2 ml, 2 mg protein) were carefully layered on top of a 30–7% (g/100 ml) linear sucrose gradient, and centrifugation was performed for 270 min at 130,000 *g* and 4° in a Beckman swinging bucket rotor. Twenty fractions were collected with a gradient collector. The amount of protein and (Na^+, K^+)-ATPase specific activities were measured in each fraction to choose which ones would be used for phosphorylation and SDS–polyacrylamide gel electrophoresis. Protein concentrations were determined by the method of Bradford [17], using a Bio-Rad protein assay (standard procedure) with bovine serum albumin for standardization.

Phosphorylation of (Na^+, K^+)-*ATPase.* Partially purified (Na^+, K^+)-ATPase was incubated with 3–5 μM [^{32}P]ATP (10 μCi/tube), 3 mM $MgCl_2$, 30 mM Tris–HCl (pH 7.1), 140 mM NaCl, with or without 3 mM KCl and varying concentrations of PHT in a total volume of 100 μl at room temperature. The reaction was initiated by addition of [^{32}P]ATP and stopped after 10 sec with trichloroacetic acid. After two trichloroacetic acid washes and two water washes, the samples were treated with 1 mM EDTA and saturated phenylmethylsulfonylfluoride solution before addition of Laemmli sample buffer [18].

SDS–gel electrophoresis. The polypeptide components of the partially purified enzyme were resolved in a discontinuous polyacrylamide gel electrophoretic system, using a slab gel apparatus with cooler, as described by Laemmli [18]. Gels were prepared from a stock solution of 30% (by wt) acrylamide and 0.8% (by wt) *N,N'*-bis-methylene acry-

lamide. The separating gel contained 6% acrylamide, 0.375 M Tris–HCl (pH 8.8), 0.1% SDS, and 0.1% ammonium persulfate. The stacking gel contained 3% acrylamide, 0.125 M Tris–HCl (pH 6.8), 0.1% SDS, and 0.1% ammonium persulfate. The running buffer consisted of 0.025 M Tris–HCl (pH 8.3), 0.19 M glycine, and 0.1% SDS. Molecular weight markers were phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400).

Gel electrophoresis was performed at 4° under constant current of 20 mA/gel until the migration front went into the separating gel and then 40 mA/gel for 3–4 hr. The gels were stained with silver (Bio-Rad silver staining kit), dried on cellophane sheets, and then placed in Kodak cassettes (with intensifying screens) against Kodak X-ray films for a period of up to 10 days at −70°.

Data analysis. The developed autoradiographs were analysed by an LKB ultrascan laser densitometer connected to an analytical integrator (Hewlett-Packard) programmed to identify density peaks and derive the area. The effect of K^+ and PHT was studied by determining the percentage of change from the reference phosphorylation level (5 μM ATP, 3 mM MgCl2, 30 mM Tris–HCl, pH 7.1, 140 mM NaCl). Statistical significance of the results was calculated by standard or paired Student's *t*-test of absolute levels of densitometric units.

Assay of (Na^+, K^+)-*ATPase activity.* According to the procedure described by Medzihradsky *et al.* [19] (Na^+, K^+)-ATPase activity was determined at 37° in a medium containing 10 mM Tris (pH 7.4), 5 mM MgCl2, 125 mM NaCl, 3 mM KCl, and 5 mM disodium ATP. (Na^+, K^+)-ATPase activity was defined as that activity inhibited by 1 mM ouabain. After a preincubation period of 5 min, the reaction was initiated by addition of ATP. The reaction was stopped after 60 min by addition of $HClO_4$. P_i content was estimated by a spectrophotometric procedure using sulfuric acid, ammonium molybdate, and ferrous sulfate [19].

Materials. PHT was obtained from Parke-Davis (sodium diphenylhydantoinate). PHT was prepared in specified concentrations by partial solubilization in the reference reaction mixture. The final concentrations were checked by fluorescence polarization. Concentrations of 10^{-4}–10^{-10} M PHT did not alter the pH of the incubation mixture. Tris–ATP, disodium ATP, and ouabain were from Sigma Chemical Co (St Louis, MO), All chemicals were analytical grade. Chemicals and molecular weight standards for electrophoresis were from Bio-Rad Laboratories (Richmond, CA). The [^{32}P]ATP came from New England Nuclear (Boston, MA) (20–30 Ci/mmol).

RESULTS

Purification of brain (Na^+, K^+)-*ATPase by SDS-extraction*

Table 1 indicates wet weights of starting material and protein contents of microsomal fraction. After centrifugation and collection, the gradient fractions with maximal (Na^+, K^+)-ATPase specific activities had a protein concentration of ±0.2 mg/ml. They were used for phosphorylation and electrophoresis experiments. After partial purification, maximal (Na^+, K^+)-ATPase specific activities measured 118 ± 19 μmol P_i/mg protein/hr in mice and lower values in cats and human cortex (Table 1). These corresponded to an approximate purification of 54-fold [ratio of (Na^+, K^+)-ATPase specific activities in the gradient fraction and cortex homogenate] with a yield of ±6% (ratio of total ATPase activities in the gradient fraction and in the cortex homogenate) in mice, somewhat higher than in cats and humans.

After phosphorylation, 5–15 μg of microsomal proteins were analysed by SDS–polyacrylamide gel electrophoresis, followed by autoradiography. The most constantly stained bands were two closely spaced bands at MW ± 100,000 representing 27, 20 and 15% of the total staining of the gel, respectively in mice, cats and human cortex. As identified by its unique phosphorylation characteristics (see below), the 100 Kd doublet is the catalytic subunit of the (Na^+, K^+)-ATPase; the alpha(+) (the higher MW form) and the alpha(−) (the lower MW form) subspecies were clearly separated on 6% acrylamide gels (Fig. 1). After molecular weight calibration with protein standards on 6% SDS-gels, alpha(+) and alpha(−) appeared to have calculated MW of 102,000 and 97,000; 100,000 and 93,000; 102,000 and 94,000 respectively in mice, cats and human cortex. Thus, the resolution of those two subunits in cats was better than in mice, but lower than in humans. The incubation conditions (absence or presence of K^+, PHT) did not alter the electrophoretic mobility of (Na^+, K^+)-ATPase catalytic subunits.

Phosphorylation of (Na^+, K^+) *-ATPase*

Sodium-stimulated phosphorylation and potassium-induced dephosphorylation are unique characteristics of the catalytic subunit of purified (Na^+, K^+)-ATPase. This enabled us to localize the subunit on a stained gel and its corresponding autoradiogram. Both the alpha(+) and alpha(−) bands were phosphorylated in the presence of Na^+ and dephosphorylated by K^+ (Fig. 1). However, as mentioned above, the resolution of these two bands on the gel was not identical in the three species and autoradiograms showed the two bands as two overlapping entities, picked up as one band by the densitometer beam, more often in mice than in cats and humans. Hence, results will first deal with the effect of PHT on the net phosphorylation of the global catalytic subunit and then with regard to differential effects on alpha(−) and alpha(+) subspecies in cat and human cortex.

Effect of PHT on the phosphorylation of (Na^+, K^+)-*ATPase catalytic subunits*

We previously showed the dephosphorylating activity of PHT on the catalytic subunit of (Na^+, K^+)-ATPase in C57/BL mice [20].

At 10^{-4} M, PHT decreased the net phosphorylation level of the alpha subunit in the presence of 140 mM Na^+ by ±50% in C57/BL mice. By comparison, 3 mM K^+ decreased that level by more than 90%. A those concentrations, the effect of K^+ is significantly larger than the effect of PHT ($P < 0.01$).

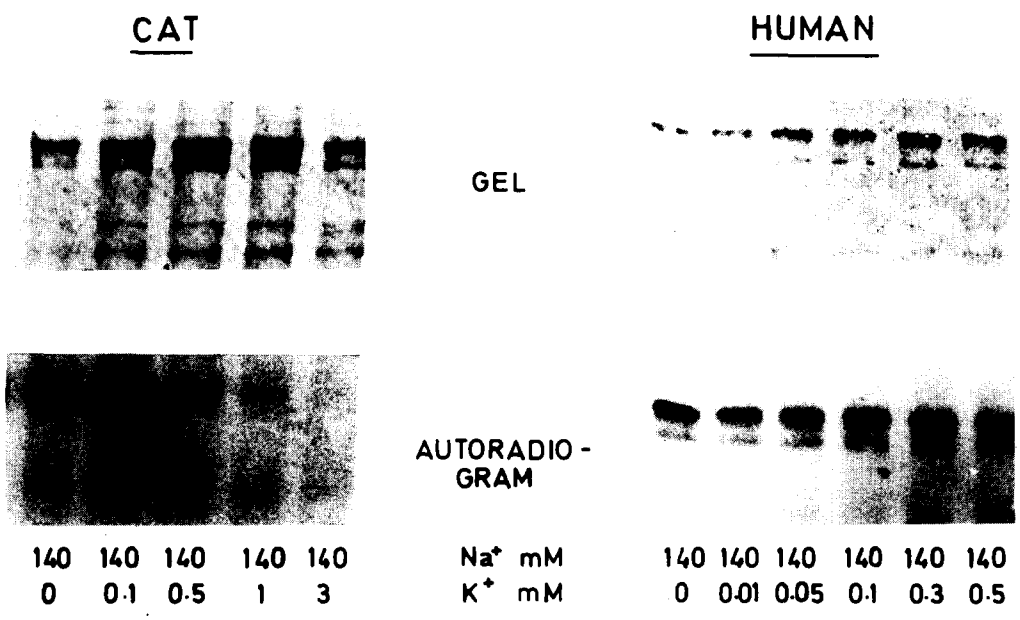

Fig. 1. SDS–polyacrylamide gel and autoradiograms after electrophoresis of partially purified (Na^+, K^+)-ATPase from cat and human cortex. The different lanes correspond to various concentrations of K^+ (from O to 1 mM). Note that the two closely spaced bands around 100 Kd, namely the alpha(+) and alpha(−) subspecies of the catalytic subunit, are dephosphorylated in presence of increasing concentrations of K^+ ions.

Table 2. Effect of 3 mM K^+ and 0.1 mM PHT on the phosphorylation level of the (Na^+, K^+)-ATPase catalytic subunit in C57/BL mice, cats and humans cortex

	C57/BL mice	Cats	Humans
3 mM K^+	2 ± 4%	4 ± 4%	3 ± 2%
	N = 9	N = 3	N = 4
	P < 0.005	P < 0.005	P < 0.005
0.1 mM PHT	47 ± 21%	55 ± 10	67 ± 6%
	N = 9	N = 3	N = 4
	P < 0.005	P < 0.005	P < 0.05

Results are expressed as percentages of the reference phosphorylation level measured in 3 mM $MgCl_2$, 30 mM Tris–HCl (pH 7.1), 140 mM NaCl and 5 μM ATP. Data are mean ± SD values. Statistical significance was determined by paired Student's *t*-test of absolute levels of densitometric units in experimental and reference conditions.

Similar results were obtained in normal cat and human cortex (Table 2). The dose dependence of the observed effect in C57/BL mice and cats cortex is shown by Fig. 2. It disappears at PHT concentrations below 10^{-7} M.

In mice cortex, we were not able to separately study the phosphorylation level of alpha(+) and alpha(−) subunits, because of their poor resolution on the autoradiograms. In cat and human cortex, where the resolution between the two subunits was much better (Fig. 1), we found that phenytoin decreased the phosphorylation level of the alpha(−) subunit at a greater extent ($P < 0.05$) than of the alpha(+) subunit (Table 3). IC_{50} values were calculated at 3.5×10^{-6}, 7.4×10^{-6} and 3.6×10^{-6}, 8.8×10^{-6} mM PHT respectively for alpha(−) and alpha(+) in cat and human cortex.

Effect of PHT on the specific activity of microsomal (Na^+, K^+)-ATPase

We tested the influence of PHT (10^{-4} M) on the specific activity of microsomal (Na^+, K^+)-ATPase purified from mice, cats and human cortex. In the presence of 125 mM NaCl and 3 mM KCl (see Materials and Methods), PHT was unable to modify the enzymatic specific activity (Table 4), whatever the preincubation time (0–60 min) was.

DISCUSSION

In recent years, many hypotheses for the anti-epileptic action of PHT have been proposed: stimulation of (Na^+, K^+)-ATPase and the [Na^+, K^+] pump, blockade of passive Na^+ influx, blockade of voltage-sensitive sodium channel, blockade of calcium influx, enhancement of chloride-mediated inhibitory post-synaptic potentials, inhibition of protein phosphorylation and neurotransmitter release, and possible interactions with membrane phospholipids and secondary effects on membrane-bound enzymes (Ref. 13 for review).

Among those, two have been advanced to explain the observed decrease of intracellular sodium. The first hypothesis proposed that phenytoin facilitates active transport by activating (Na^+, K^+)-ATPase [11, 13, 21–23]; the second proposed that PHT limits downhill inward sodium movement during depolarization [16, 24]. The mechanism by which PHT reduces sodium conductance is not known, but it is believed that it may be linked to a primary action on the calcium control of neuronal activity [16]. The first hypothesis has been very controversial, because although such a mechanism could indeed explain the

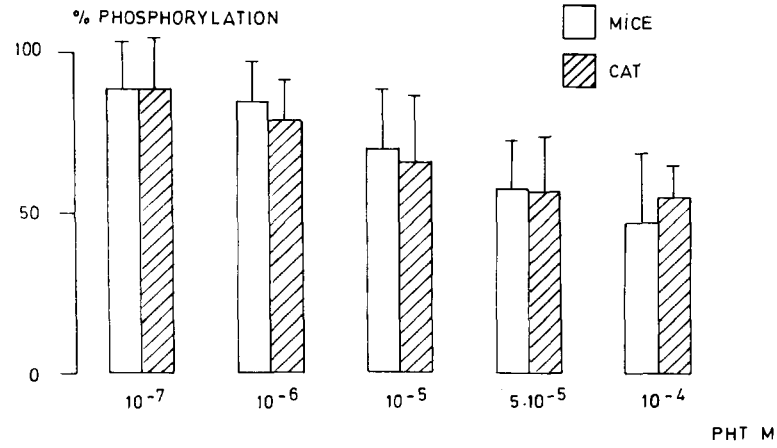

Fig. 2. Effect of PHT (10^{-7}–10^{-4} M) on the phosphorylation level of the brain (Na^+, K^+)-ATPase total catalytic subunit in mice (open bars) and cats (hatched bars) cortex. The partially purified enzyme was preincubated with different concentrations of PHT and phosphorylated as described in Materials and Methods. Data are expressed as percentages of control values (without PHT) and represent mean ± SD (bars) values from at least three different experiments.

Table 3. Effect of 0.1 mM PHT on the phosphorylation level of (Na^+ K^+)-ATPase catalytic subunits in cats and humans cortex

	Alpha(−)	Alpha(+)	P value
Cat cortex	40 ± 11	62 ± 13	$P < 0.05$
Human cortex	42 ± 9	76 ± 5	$P < 0.05$

Results are expressed as percentages of the phosphorylation level measured in reference conditions. Data are mean ± SD values of three determinations. Statistical significance was determined by paired Student's *t*-test of absolute levels of densitometric units.

Table 4. Effect of 0.1 mM PHT on the specific activities of purified (Na^+ K^+)-ATPase from C57/BL mice, cats and humans cortex

	Control	+0.1 mM PHT
C57/BL mice	118 ± 19	125 ± 47
	N = 9	N = 3
Cats	84 ± 14	82 ± 20
	N = 3	N = 3
Humans	60 ± 24	72 ± 17
	N = 4	N = 4

Specific activities of (Na^+, K^+)-ATPase are expressed in μmol P_i/mg protein/hr. PHT has no significant effect in mice, cats or human cortex.

reduction of intracellular sodium, it never received experimental confirmation by studying the direct influence of PHT on (Na^+, K^+)-ATPase, except under specific experimental conditions [21]. Those studies have indeed shown conflicting results: no effects [21, 23, 25–27], stimulation [21–23, 28, 29], or even inhibition of the enzyme [21, 26, 29, 30].

We have previously shown that PHT induces a dephosphorylation of brain (Na^+, K^+)-ATPase in C57/BL mice. We already discussed the apparent discrepancy between that effect and the absence of stimulation of the enzymatic specific activity [20]. Numerous discrepancies exist in literature concerning the absolute values of (Na^+, K^+)-ATPase specific activities, depending on the technical variations in determining that activity (Na^+ and K^+ concentrations of the reaction medium, type of tissue or subcellular preparation). The specific activity of (Na^+, K^+)-ATPase measured at one Na^+ and K^+ concentration in a brain preparation does not totally reflect the real ability of the enzymatic system to pump ions across neuronal and glial membranes; it is mostly related to the energy consumption of the pump (expressed in μmol of P_i/mg protein/hr). From our point of view, it is more informative to investigate the effect of PHT on (Na^+, K^+)-ATPase phosphorylation cycle, which is known to be closely linked to the translocation of Na^+ and K^+ ions [1, 2]. We assessed that PHT is specifically acting on the dephosphorylation step of the reaction cycle. According to the sequential model of Albers [1], the activation of dephosphorylation increases the net influx of K^+ ions into the cell and accelerates the reaction cycle and therefore the transmembranar Na^+ and K^+ transport. The absence of stimulation by PHT of the (Na^+, K^+)-ATPase specific activity could also suggest a better physiological efficiency of the pump in the presence of PHT (increased ionic transport without increase of ATP consumption). We now report that PHT acts on (Na^+, K^+)-ATPase by decreasing its phosphorylation level not only in C57/BL mice, but also in normal cat and human cortex. To our knowledge there is no other report of PHT studies on human brain tissue.

Differences in (Na^+, K^+)-ATPase specific activities were observed between mice, cat and human cortex (Table 1). In human cortex, the enzymatic activity was the lowest, around half of the value measured in mice cortex. Those differences could be related to species differences, but the influence of anaesthesia, hypoxia and drugs may be involved. In spite of those differences in baseline activities, the experiments have shown a significant, reproducible

influence of phenytoin, although differences in the extent of the PHT dephosphorylating effects were also observed (Table 2).

Our results have also confirmed the existence of two molecular forms of (Na^+, K^+)-ATPase, based on their resolution by SDS–gel electrophoresis. The difference in molecular weight between those two subspecies is greater in human cortex when compared to cat and mice cerebral cortex, suggesting species differences in the amino acid sequence of both subunits. That is why an accurate measurement of the effect of PHT on their respective phosphorylation levels was relatively easier for human tissue compared to mouse or cat tissue.

Our data indicate that the dephosphorylating influence of PHT is greater on the alpha(−) subunit, which is known to be predominant in non neuronal cells [4]. The results however show that PHT has a significant dephosphorylating effect on neuronal, alpha(+) (Na^+, K^+)-ATPase. Recently, White *et al.* [14] showed that chronic PHT enhances the activity or increases the synthesis of (Na^+, K^+)-ATPase in glial fraction. As these authors point out, it is possible that the discrepancies in the literature regarding the influence of PHT on (Na^+, K^+)-ATPase specific activity can be explained by the lack of studies on brain subcellular fractions. On glial cell cultures, the same authors showed that PHT increased efficiency of (Na^+, K^+)-ATPase over a wide range of K^+ concentrations [14]. Together with White's data, our results support the hypothesis that PHT stimulates the glial (Na^+, K^+)-ATPase, enhancing the ability of brain tissue to regulate extracellular concentrations of K^+ ions. Since abnormal K^+ regulation has been suggested to play a role in the ictal transition and seizure spread from an epileptogenic focus, but not in the initiation of interictal discharges [31–34], PHT's ability to enhance K^+ uptake within glial and neuronal cells could be effective when interictal discharges are being transformed to ictal episodes. Electrophysiological studies have indeed shown that PHT is effective in reducing the spread of seizure discharge in all areas from its focus, but has only a selective effect on seizure threshold [35, 36]. Those observations correlate well with clinical aspects of PHT use in epileptic patients: the drug is very active in preventing secondary generalization from partial seizures, but does not completely eliminate the prodromal signs nor the epileptic paroxysms in the seizure focus itself [37].

The results presented in this report provide new evidence on how therapeutic concentrations (10^{-4}–10^{-7} M) of PHT could stimulate active Na^+ and K^+ transport across glial membranes. Conflicting electrophysiological and biochemical data are not exclusive, since all electrophysiological studies were performed on neuronal preparations and most biochemical studies were done on homogenates or microsomal fractions (containing both neuronal and glial membranes). It is therefore possible that PHT stimulates (Na^+, K^+)-ATPase in glial cells and blocks voltage-sensitive Na^+ channels in neuronal cells. Additional electrophysiological and biochemical studies are needed to better understand the regulatory processes of glial cells.

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