# Regulation of the frontocortical sodium pump by $Na^+$ in Alzheimer's disease: difference from the age-matched control but similarity to the rat model

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**Abstract The $Na^+$ and $K^+$ dependence of the frontocortical Na,K-ATPase in Alzheimer's disease (AD) was compared with that in human control (Co) and rat AD model. In AD, the relationship between the Na/K ratio and the Na,K-ATPase activity showed noticeable left-shift with three-fold increase in the enzyme affinity for $Na^+$ ($K_{0.5}$ = 10 and 30 mM in AD and Co, respectively). The $Na^+$ dependence of the enzyme in AD showed two different Hill coefficients ($n_H$), 1.1 and 0.3, whereas the Co value of $n_H$ was higher (1.4). The rat AD model generated by ibotenic acid revealed a $Na^+$ dependence similar to AD. The $K^+$ dependence of the Na,K-ATPase showed no significant difference in AD and Co. Compared with Co, AD produced a shift in the break of the Na,K-ATPase Arrhenius plot, suggesting remarkable alterations in the enzyme lipid environment. Our findings support the hypothesis that dysfunction of the Na,K-ATPase in AD is provoked by altered $Na^+$ dependence of the enzyme. An impairment of the pump functionality might serve as an early mechanism of AD that should be interrupted by selective pharmacological agents.**


*Key words:* Alzheimer's disease; Na,K-ATPase; $Na^+$ dependence; Conformational transition; Lipid environment

## 1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder, characterized by a complex of neuronal abnormalities. Premature loss of neurons in AD has been shown to be associated with an oxidative stress, impairment of energy metabolism, membrane dysfunction, disorder of signal transduction, disruption of ionic homeostasis and with other particular mechanisms [1–3]. Previous studies have shown the impairment in AD brain of the membrane-bound Na,K-ATPase – the sodium pump mediating the active transmembrane transport of $Na^+$ and $K^+$ [4,5]. On the basis of the data obtained in the human hippocampal synaptosomes and in the cultured rat hippocampal neurons, it was suggested that the reduction in the Na,K-ATPase activity is caused by the neurotoxic forms of amyloid-β peptides (Aβ) [6]. Aβ have been shown to induce membrane lipid peroxidation in the cultured neurons, a process which might lead to the impairment of ion-motive ATPases, including Na,K-ATPase [7]. However, a detailed molecular mechanism underlying the dysfunction of Na,K-ATPase in the brain of AD patients remains unclear. The changes in the binding of $Na^+$ and $K^+$ to the Na,K-ATPase followed by the enzyme downregulation and the elevation of the intracellular ratio of $Na^+$ and $K^+$ ($Na_i/K_i$) appear to be implicated in several pathologic conditions, such as hypertension, tumor genesis, cardiac insufficiency etc. [8]. In this context, it would be reasonable to suppose that altered binding properties of $Na^+$ and $K^+$ to the Na-pump might be related to the genesis of neuronal dysfunction in AD. To test this, we compared the specific activity and the $Na^+$ and $K^+$ dependence of the Na,K-ATPase in the membranes from AD and age-matched control frontal cortices, one of the more injured regions in the AD brain. In parallel, the same parameters were estimated for the frontocortical Na,K-ATPase from the rat AD model generated by ibotenic acid. To elucidate whether the alterations in the regulation of Na,K-ATPase by univalent cations could be related to the altered protein–lipid interactions of the enzyme, we determined the temperature (Arrhenius) dependence of Na,K-ATPase in both, AD and control frontocortical membranes.

## 2. Materials and methods

### 2.1. Enzyme preparation and assay

Postmortal tissues from the frontal cortex obtained at autopsy from six AD patients (age, 85 ± 4 years) and from six age-matched nondemented control subjects (age, 78 ± 6 years) were used in this study. All AD cases exhibited senile plaques, neurofibrillary tangles and other criteria of AD [9], while control brains showed no neuropathological changes. Immediately after autopsy, the brain tissues were stored at −75°C until thawing for isolation of Na,K-ATPase preparations.

In the parallel experiments, the frontocortical tissue from the rat AD model was used. The model was generated by bilateral lesion of the Meynert's basal magnocellular nuclei in male Wistar rats (240–260 g) by injecting 0.75 μl of ibotenic acid (10 μg/ml) [10]. The rats were killed by decapitation, the frontocortical areas dissected, and Na,K-ATPase preparations isolated.

In all cases, the preparations of Na,K-ATPase were isolated according to the method described earlier [11]. The tissues were homogenized at 4°C in medium containing 0.32 M sucrose, 1 mM EDTA, 0.1% DOC (deoxycholate) and 37.5 mM imidazole–HCl (pH 7.4 at 8°C). The homogenate was centrifuged for 10 min at 10 000×*g* and the

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clear supernatant was centrifuged for 30 min at 24 000×*g*. The enzyme preparation was achieved by resuspension of final sediment in the above-described buffer (without DOC). The total ATPase activity was measured by the incubation of the membrane protein (10–20 μg) in 375 μl of medium containing 100 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 4 mM Tris–ATP and 25 mM imidazole–HCl (pH 7.4 at 37°C). Variations in incubation conditions are detailed in the appropriate figure legends. The reaction was terminated after 7 min and inorganic phosphate ($P_i$) was determined according to [11]. The ouabain-sensitive Na,K-ATPase activity was found as the difference between the release of $P_i$ from ATP in the absence or presence of 10–100 μM ouabain in the incubation medium. The protein content in the enzyme preparation was determined by the method of Lowry et al. [12], using bovine serum albumin as a standard. Under all experimental conditions, the activity of Na,K-ATPase was linear as a function of incubation time and enzyme amount.

### 2.2. Other measurements

The relationship between the $Na^+/K^+$ ratio and the Na,K-ATPase activity was measured at a constant combined concentration of $Na^+$ and $K^+$ (150 mM) in the incubation media. The Hill coefficients (the degree of cooperativity, $n_H$) and $K_{0.5}$ (the sodium or potassium concentration at which the enzyme reaches half of its maximal activity) for control and AD Na,K-ATPase were determined by the method described earlier [13]. The values of $n_H$ and $K_{0.5}$ were calculated from the Hill plots {log ($v/V_{max}-v$) vs. log [$Na^+$] or log [$K^+$]}, using the GraphPad Prizm program (version 3.0). The temperature (Arrhenius) dependence of Na,K-ATPase was studied between 13 and 37°C and the data were plotted as log $v_0$ vs. temperature $t$.

All the experiments were repeated at least three times and means ± S.E.M. were calculated. The unpaired Student $t$-test was used to identify significant differences ($P < 0.05$) between control and AD group.

## 3. Results

Our first finding was that the specific activity of the Na,K-ATPase in AD frontal cortex (92 ± 6 nmol $P_i$/min/mg protein) was significantly ($P < 0.05$) lower than that in the age-matched control (151 ± 30). Similarly, the frontocortical Na,K-ATPase activity in the ibotenate-generated rat AD model (690 ± 10 nmol $P_i$/min/mg protein) had significantly decreased from the value of the rat control (1160 ± 110).

Fig. 1 indicates that in the control, the relationship between the frontocortical Na,K-ATPase activity and the Na/K ratio in the reaction mixture was significantly different from that in AD. While the control revealed maximal activation of the enzyme at 130 mM concentration of $Na^+$ ($K^+$ concentration

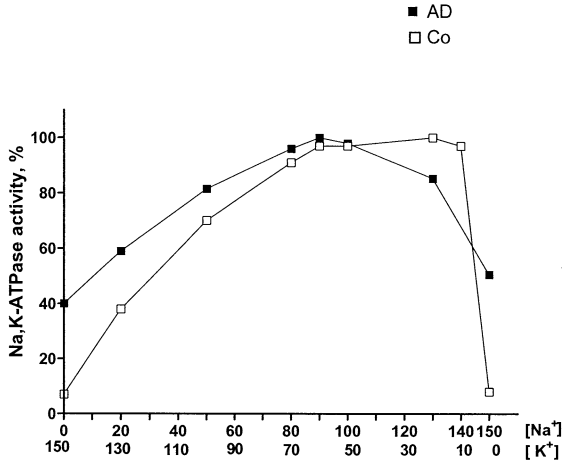


Fig. 1. Na/K dependence of the Na,K-ATPase activity in the membranes from Alzheimer's disease (AD) and control (Co) frontal cortex (% from maximal activity; a typical experiment in duplicate).

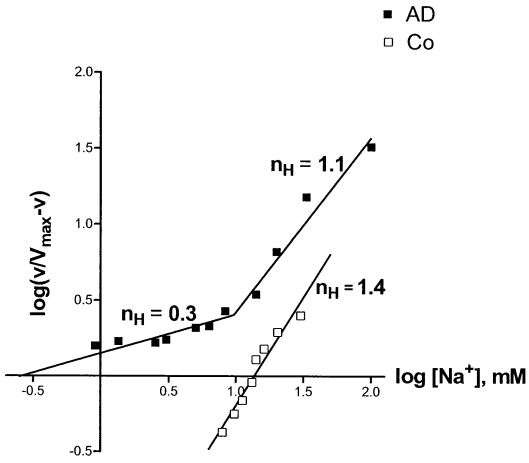


Fig. 2. Hill plots for the $Na^+$ dependence of the Na,K-ATPase activity in the membranes from AD and Co frontal cortex (a typical experiment in duplicate). The Hill coefficient ($n_H$) was obtained from the slope.

20 mM, respectively), in AD, the parameter showed a significant ($P < 0.05$) left-shift to the $Na^+$ range of 80–100 mM (at 70–50 mM of $K^+$). In the control, the half-maximal activation ($K_{0.5}$) of the enzyme by $Na^+$ was characterized by a mean value of 29.5 ± 0.9 mM, whereas the same parameter for AD was 8.7 ± 0.7 mM. Therefore, a three-fold increase in the affinity of the enzyme to $Na^+$ was observed in AD compared with the control.

At a fixed basic concentration of $K^+$ (20 mM), the $Na^+$ dependence of the Na,K-ATPase in AD frontal cortex showed an interesting difference from that in the control (Fig. 2). In AD, we could discriminate between two types of cooperative $Na^+$ effects with the Hill coefficient ($n_H$) values of 1.1 ($K_{0.5}$ = 4.2 mM) and 0.3 ($K_{0.5}$ = 0.2 mM), respectively. At the same time, the control region revealed one linear Hill plot with $n_H$ of 1.4 ($K_{0.5}$ = 13.3 mM). The $Na^+$ dependence of the frontocortical Na,K-ATPase in the rat AD model revealed characteristics quite similar to AD. As shown in Fig. 3, the bilateral infusion of ibothenic acid induced $Na^+$ effects with two $n_H$ values, whereas in control rats the effect showed one value of $n_H$. In comparing the $K^+$ dependence of the Na,K-ATPase (at 100 mM of $Na^+$) in AD and human control fron-

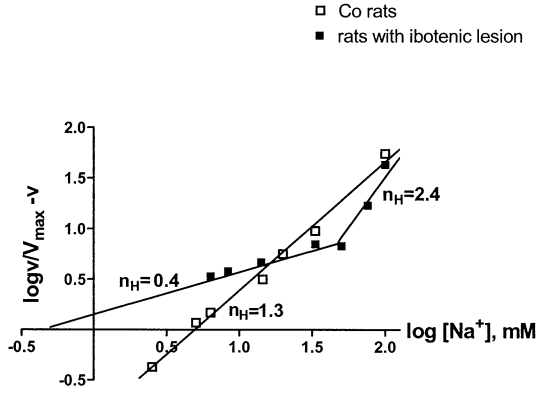


Fig. 3. Hill plots for the $Na^+$ dependence of the Na,K-ATPase activity in the frontocortical membranes from control (Co) rats and from rats with ibotenic acid lesion (a typical experiment in duplicate).

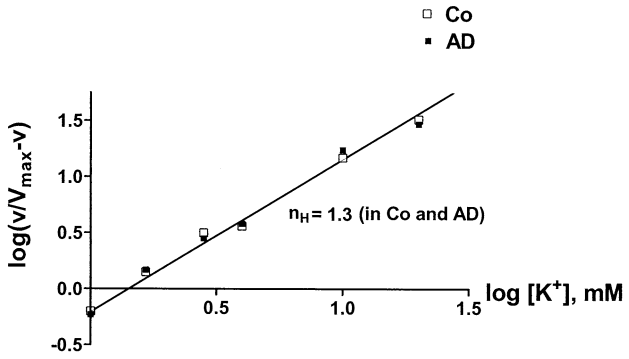


Fig. 4. Hill plots for the $K^+$ dependence of the Na,K-ATPase activity in the membranes from AD and Co frontal cortex (a typical experiment in duplicate). The Hill coefficient ($n_H$) was obtained from the slope.

tal cortex, no significant changes were observed for the values of $n_H$ or $K_{0.5}$ (Fig. 4). Similarly, the $K^+$ parameters of the Na,K-ATPase showed no difference between the ibotenic acid-injured and the control rat frontal cortex (data not shown).

In the membranes from the human control frontal cortex, the Arrhenius plot for the Na,K-ATPase activity revealed a break at the position of 21.6°C (Fig. 5). In AD frontal cortex, the break value increased by 6.4°C above the control value, indicating that AD induces phasic transitions in the membrane lipid microenvironment of the Na,K-ATPase.

## 4. Discussion

The pathogenesis of AD is supposed to involve multiple factors, including neuronal membrane disorders. The present study and previous findings [14] have demonstrated reduced activity of the membrane-bound Na,K-ATPase (Na-pump) in the more injured regions of AD brain, suggesting that the enzyme dysfunction might predispose or accelerate AD progression. However, the mechanism (probably changes in ion transport parameters) underlying the dysfunction of Na,K-ATPase in AD is not clear yet.

Our results showed a significant left-shift in the Na/K ratio for the maximum Na-pump stimulation in AD compared with the control. Such a shift, together with AD-induced increase in the $Na^+$ affinity, provides evidence for the alteration in the relative quantity of the Na,K-ATPase conformational forms, $E_1Na$ and $E_2K$, in favor of the $E_1Na$ form, in which the enzyme has been shown to bind preferentially intracellular sodium [13,15]. The conformational transition of the Na-pump in AD might be evaluated as an adaptive response to the reduced $Na^+$ cooperativity, a phenomenon we could realize through the Hill coefficient decrease and Hill plot break for $Na^+$ in AD. In addition, the expression and association heterogeneity of α- and β-subunits for the multiple isoforms of Na,K-ATPase have recently been demonstrated [16]. Since the prevalent isozyme in the brain, $\alpha_3\beta_2$, has relatively high affinity for $Na^+$ [16,17] and since the Na,K-ATPase of AD frontal cortex reveals markedly higher $Na^+$ affinity than the control, it might be suggested that more injured AD brain regions are capable of actively expressing the $\alpha_3\beta_2$ and other Na-affine isozymes essential for maintaining the regional ionic homeostasis. It is interesting that cooperativity in $K^+$ binding showed no significant difference in control and AD frontal cortex. Hence, binding of $K^+$ to the Na,K-ATPase, mainly occurring at the enzyme extracellular side [15], seems to be less susceptible to AD-inducible impairment than binding of $Na^+$ to the intracellular side. Supporting evidence for our data has been obtained in the experiments with the rat AD model. Under the conditions of basal forebrain ibotenic lesion the rat frontocortical Na-pump showed instability with respect to the $Na^+$ cooperative binding, while the cooperativity for $K^+$ was preserved. Taken together, these results coincide with the previous assumption that certain pathological conditions in the brain, such as tumorigenesis, may interfere with the $Na^+$ cooperative binding to the Na,K-ATPase and shift the balance between the conformational forms of the enzyme [18].

This study revealed a noticeable shift of the break point in the Na,K-ATPase Arrhenius plot in AD frontal cortex compared with the control, suggesting certain alterations in the membrane lipid microenvironment (the phase transitions, the changes of the hydrophobic volume etc.) of the enzyme. Because the Na,K-ATPase is one of the targets for the neuronal oxidative stress [19] and because severe oxidative stress is directly involved in the pathogenesis of AD [20,21], it might be postulated that the enzyme lipid environment is directly modified by AD-induced high-level lipid peroxidation. Several authors have shown that alterations in the neuronal Na,K-ATPase lipid environment in the pathological and experimental conditions lead to tighter interaction of the enzyme with the lipid bilayer [11,16]. This in turn may interfere with protomeric interaction in the oligomeric enzyme protein, resulting in the decline of its cooperativity for cations, nucleotides and other regulatory ligands [18,22]. According to our viewpoint, the altered lipid–protein interactions we were detecting for the neuronal Na,K-ATPase in AD may underlie the Na-cooperativity failure of the enzyme and its transition to the Na-form. Impaired $Na^+$ transport by the Na,K-ATPase reportedly leads to an increase in the intraneuronal sodium and, through the elevated Na/Ca exchange, in the calcium [23]. In turn, abnormalities in the calcium dynamics are known to contribute to neuronal apoptosis and degeneration [24,25].

In summary, our findings support the hypothesis that altered binding of $Na^+$ to the Na,K-ATPase is a substantial

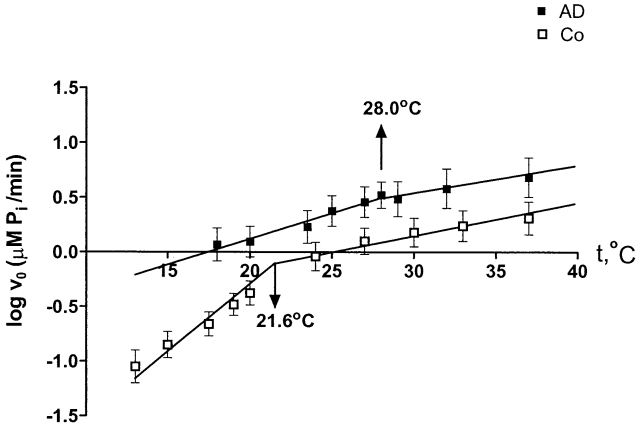


Fig. 5. Temperature (Arrhenius) dependence of the Na,K-ATPase activity in the membranes from AD and Co frontal cortex ($v_0$: velocity of the enzyme reaction). Values represent means ± S.E.M., $n = 3$ per group.

mechanism leading to the dysfunction and the activity alteration of the enzyme in AD brain. Due to the significance of the Na-pump for the maintenance of the physiological $Na^+$ and $K^+$ gradients, an impairment of the pump functionality may be considered as one of the early events accelerating the progression of AD, particularly in the more affected brain regions. The fact that some of the functional alterations in the Na-pump are reversible [26] reinforces the importance of developing diagnostic tools for the detection of the Na-pump kinetic parameters and conformational changes in the early (subclinical) stages of AD. On the other hand, the design and use of pharmacological agents that can successfully interrupt the Na-pump-mediated pathologic events in AD brain should offer a good chance for controlling the progression of the disease.

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