Biochemical Pharmacology, Vol. 34, No. 24, pp. 4197–4201, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00

CHARACTERIZATION OF THE Na^+,K^+-ATPase ACTIVITY OF BASOLATERAL PLASMA MEMBRANES OF KIDNEY PROXIMAL TUBULAR CELLS FROM YOUNG AND OLD RATS

REINALDO MARÍN, TERESA PROVERBIO and FULGENCIO PROVERBIO*
Instituto Venezolano de Investigaciones Cientificas (IVIC), Centro de Biofísica y Bioquímica, Caracas 1010-A, Venezuela

(*Received* 28 *December* 1984; *accepted* 16 *April* 1985)

Abstract—Several characteristics of the Na^+,K^+-ATPase activity of basolateral plasma membranes of kidney proximal tubular cells from young (3 months) and old (24 months) rats were studied. In both cases, the ATPase activity reached optimum values under the following conditions: Mg^{2+}:ATP concentrations (mM) 5:5 (apparent K_m 0.5 mM); Na^+ concentration 50 mM (apparent K_m 18 mM); K^+ concentration 20 mM (apparent K_m 2.5 mM); pH 7.2; temperature 52°. The values of the apparent energy of activation of the system were similar for young and old rats in the temperature range 20–52° but were 55% higher for the old rats in the temperature range 10–20°.

It has been shown that the active extrusion of Na^+ exchanged for K^+ in rat kidney cortex slices is affected by ageing [1, 2]. It has also been shown [1, 3, 4] that the ATPase system associated with this Na^+ transport, i.e. the Na^+,K^+-ATPase, is also affected by ageing.

The ratio "Na^+,K^+-ATPase activity/[^{3}H]-ouabain binding" was found to be lower for kidney membranes from old rats [1, 4]. This lower ratio is an indication that the turnover of the Na^+,K^+-ATPase is diminished by ageing. The turnover of an enzyme depends on multiple factors: substrate and ligand concentration and optimum pH and temperature values. Accordingly, it is important to compare the effects of these variables on the Na^+,K^+-ATPase from old and young rat kidneys in order to measure any age-induced changes that could explain the lower turnover rate of the Na^+,K^+-ATPase described for old rats [4].

In this work several characteristics of the Na^+,K^+-ATPase of basolateral plasma membranes from young (3 months) and old (24 months) rat kidney cortex cells were studied. It was found that ageing increased the apparent energy of activation of the Na^+,K^+-ATPase of the old rats nearly 55%, in the temperature range of 10–20°.

MATERIALS AND METHODS

Outermost slices of kidney cortex (rich in proximal tubules) of young (3 months) and old (24 months) rats were obtained as previously described [5]. Each gram of tissue was homogenized at 4° with eight strokes at 2500 rpm in an Eberbach homogenizer with a Teflon pestle, in 3 vol. of a solution of 0.25 M sucrose and 20 mM Tris-HCl (pH 7.2). The plasma membrane enriched fractions were prepared according to the described method [4, 6]. The final pellet was resuspended in the sucrose–Tris medium, frozen, and kept at −20°.

Assay of the ATPase activity. ATPase activity was determined as already described [7]. Briefly, the membrane suspensions were preincubated for 5 min, usually at 37°, and the incubation was started by the addition of ATP. The incubation medium contained (final concentrations): 50 mM Tris–HCl (pH 7.2); 5 mM $MgCl_2$; 100 mM NaCl; 20 mM KCl; and 2 mM ATP. The ouabain-insensitive ATPase activity was determined in the same medium, without K^+ and in the presence of 2 mM ouabain. The Na^+,K^+-ATPase activity was taken as the difference between the two conditions. Preliminary experiments were carried out to ascertain the linearity of the studied system as a function of the incubation time and the quantity of protein in the incubation medium. For both preparations (from young and old rats), the activity of the Na^+,K^+-ATPase was linear with the incubation time, at least, for the first 15 min. This incubation time was chosen for our experiments. On the other hand, the activity was linear within a wide range of protein in the incubation medium (20–150 μg/ml). Usually the protein concentration of the incubation tubes was 40–60 μg/ml. Vesicle formation was avoided by treating the membranes before the assays with 0.06% deoxycholate (DOC) and 2 mM EDTA at pH 7.2, according to the method of Jørgensen and Skou [8]. Data were expressed as the mean ± S.E. of the determinations. Differences between the results were analyzed according to Student's *t*-test. Significance was accepted at $P < 0.05$.

RESULTS

As previously found [1, 4], the activities of the Na^+,K^+-ATPase of kidney cortex slice homogenates

* Send correspondence to: Dr. F. Proverbio, IVIC, Centro de Biofísica y Bioquímica, Apartado 1827, Caracas 1010-A, Venezuela.

from old rats were lower than the activities of homogenates from young rats. Activity was approximately 40% lower when expressed in either nmoles P_i/mg protein·min or nmoles P_i/pmoles[^{3}H]-ouabain bound·min, indicating a lower turnover of the enzyme. The Na^+,K^+-ATPase activity of purified basolateral plasma membrane fractions prepared from the same slices was similar for both young and old rats, when expressed in nmoles P_i/mg protein·min. However, when the activity was expressed in nmoles P_i/pmole[^{3}H]-ouabain bound·min, the values for the old rats were approximately 40% lower. The fact that the activities for both purified membrane preparations (young and old rats) were the same when expressed as a function of the membrane proteins but were 40% lower for the old rats when expressed as a function of the specific [^{3}H]-ouabain binding, indicates that: (1) the basolateral plasma membrane fractions from old rats were more purified than the fractions from young rats, and (2) ageing affected the turnover of the Na^+,K^+-ATPase (for details, see Ref. 4). In the present work, the Na^+,K^+-ATPase activity is expressed in nmoles P_i/mg protein·min and in all the legends the ATPase activity values are expressed in nmoles P_i/pmole[^{3}H]-ouabain bound·min for the maximal values.

Quality and quantity of substrate. The Na^+,K^+-ATPase hydrolyzes almost exclusively ATP. Previous experiments showed that optimal Na^+,K^+-ATPase activity occurs at a Mg^{2+} : ATP ratio of 1 : 1 for the young as well as for the old rats. Consequently, we studied the effect of varying the Mg^{2+} as well as the ATP concentrations at fixed ratios of Mg^{2+} : ATP of 1 : 1. The results are shown in Fig. 1. The optimum activity was obtained, in both cases, at a Mg^{2+} and ATP concentration of 5 mM. The apparent K_m, calculated by the Woolf variation of the Lineweaver-Burk equation, was 0.5 mM, as shown in the insert.

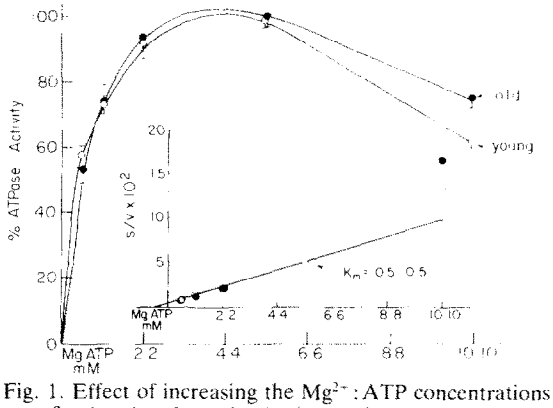

Fig. 1. Effect of increasing the Mg^{2+} : ATP concentrations at a fixed ratio of 1 : 1 in the incubation medium on the Na^+,K^+-ATPase activity of basolateral plasma membranes of kidney proximal tubular cells from young (3 months) and old (24 months) rats. One hundred percent Na^+,K^+-ATPase activity values were 7.07 ± 0.27 (young) and 4.75 ± 0.22 (old) nmoles P_i/pmoles[^{3}H]-ouabain bound·min. Values are expressed as mean ± S.E. (N = 18). In this and in the next two figures, the apparent K_m was calculated by means of the Woolf derivative of the Lineweaver–Burk transformation of the Michaelis–Menten equation of the form $S/V = K_m/V_{max} + S/V_{max}$, where $K_m = -S$ on the S axis (see insert).

Na^+ and K^+ concentrations. The effect of the Na^+ concentration on the Na^+,K^+-ATPase activity is shown in Fig. 2. The optimal Na^+ concentration (50 mM) was the same for young and old rats. The apparent K_m, as shown in the insert, was near 18 mM. Figure 3 shows the behavior of the systems toward different K^+ concentrations. In both cases, the optimum value was obtained at 20 mM K^+ and the apparent K_m, as shown in the insert, was near 2.5 mM. The K_m values were calculated by the Woolf variation of the Lineweaver–Burk equation.

Effect of ouabain. The effect of ouabain on the Na^+,K^+-ATPase activities of old and young rats was tested by incubating the membrane suspensions for 15 min at 37° in incubation medium containing $Tris^+$, Mg^{2+}, Na^+,K^+ and ATP (see Materials and Methods) with or without different concentrations of ouabain. In both cases, there was practically 100% inhibition at a concentration of 2 mM ouabain and the $K_{50\%}$ inhibition was near 0.25 mM (data not shown).

Effect of the pH of the incubation medium. In both cases (young and old rats) the optimum pH value was around 7.2. The pH values in which the Na^+,K^+-ATPase activity was 50% of the maximal activity (either below or above the optimum pH) were 6.4 and 7.7 for both young and old rats (data not shown).

Effect of the temperature of the incubation medium. Figure 4 shows the effect of the incubation temperature on the Na^+,K^+-ATPase activities of old and young rats. In both cases, the optimal temperature was around 52°. The Arrhenius plot of the data is presented in the insert. According to established criteria [9–12], the values were fixed by two straight lines: one between 10 and 20° and the other between 20 and 52°. The apparent energies of activation were calculated from the slopes of the two straight lines. Table 1 shows the apparent energies of activation, for the two ranges of temperature, for the old and young rats. As indicated by the slopes in Fig. 4, the apparent energy of activation was similar for the old and young rats for the range 20–52°, while it was 55% higher for the old rats, for the range 10–20°.

DISCUSSION

The Na^+,K^+-ATPase of basolateral plasma membranes from old rat kidney proximal tubular cells shows a lower turnover when compared with kidney membranes from young rats [1, 4]. This lower turnover cannot be explained as due to changes in affinity toward Mg^{2+} : ATP (Fig. 1), Na^+ (Fig. 2) or K^+ (Fig. 3). It was also not due to a change in the behavior of the system toward the pH of the incubation medium. There was no change with ageing in the sensibility of the system toward ouabain. The Na^+,K^+-ATPase activity for both preparations was temperature dependent (Fig. 4). Even when the optimum temperature did not change with ageing, the behavior of the Na^+,K^+-ATPase to changes of the incubation temperature below 40° apparently differed for young and old rats. The temperature curves were analyzed using an Arrhenius plot. Several authors [9–14] have

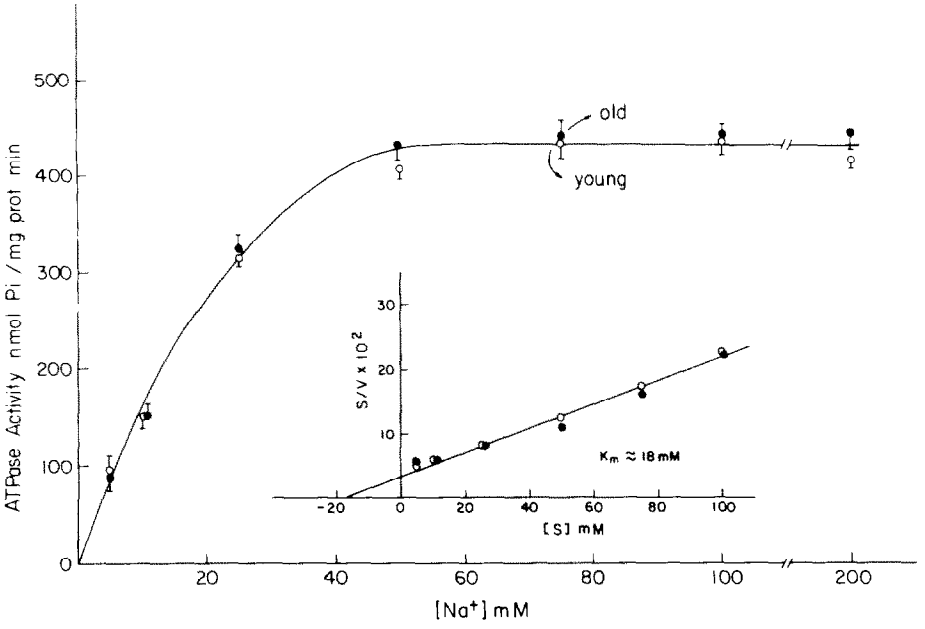

Fig. 2. Effect of increasing the Na^+ concentration (as NaCl) on the Na^+,K^+-ATPase activity of basolateral plasma membranes of kidney proximal tubular cells from young and old rats. K^+ concentration was 20 mM. Maximal activity is expressed as nmoles P_i/pmole[^{3}H]-ouabain bound·min: 7.32 ± 0.22 (young) and 4.78 ± 0.17 (old). Values are expressed as mean ± S.E. (N = 12).

shown that an Arrhenius plot of Na^+,K^+-ATPase activity between 5° and 50° yields two straight lines with totally different apparent energies of activation. The two lines intersect at the so-called transition point, which is near 20°. This transition point has been taken to be a consequence of changes in the molecular mobility of membrane lipids, in other words, in the membrane fluidity. As shown in Table 1, the apparent energy of activation of the Na^+,K^+-ATPase, calculated for the temperature range 20–52°, is similar for young and old rats, but is 55% higher for the old rats for the temperature range from 10 to 20°. These results may be due, not to a real increase in the apparent energy of activation,

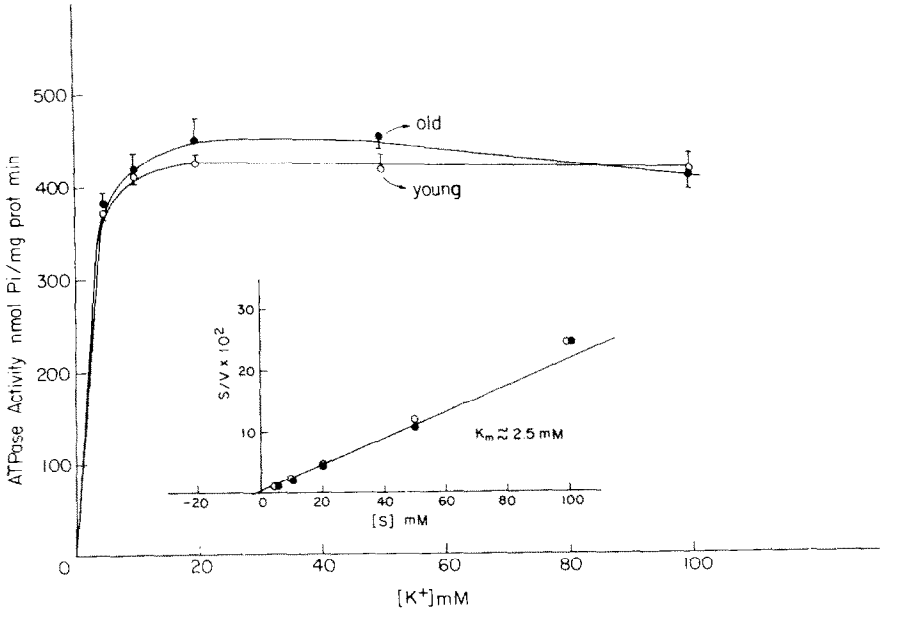

Fig. 3. Effect of increasing the K^+ concentration (as KCl) on the Na^+,K^+-ATPase activity of basolateral plasma membranes of kidney proximal tubular cells from young and old rats. Na^+ concentration was 100 mM. Maximal activity is expressed as nmoles P_i/pmole[^{3}H]-ouabain bound·min: 7.10 ± 0.27 (young) and 4.86 ± 0.22 (old). Values are expressed as mean ± S.E. (N = 12).

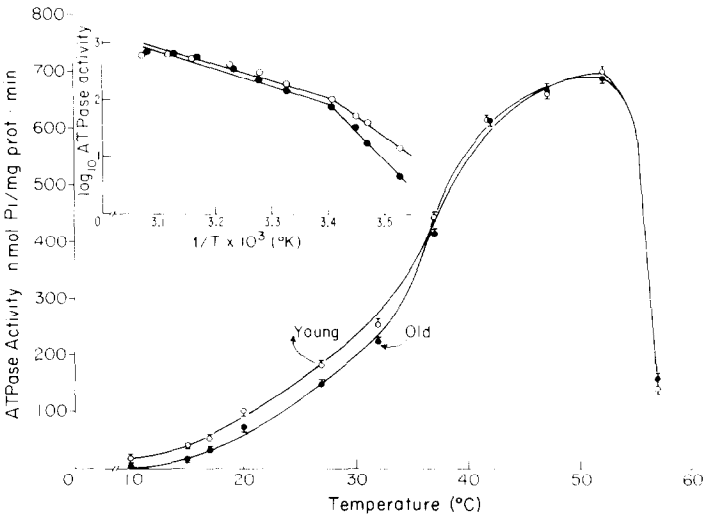

Fig. 4. Effect of the incubation temperature on the Na^+,K^+-ATPase activity of basolateral plasma membranes of kidney proximal tubular cells from young and old rats. Maximal activity is expressed as nmoles P_i/pmole[^{3}H]-ouabain bound·min: 12.00 ± 0.21 (young) and 7.42 ± 0.11 (old). Values are expressed as mean ± S.E. (N = 12). The Arrhenius plot of the data is presented in the insert.

but to an artifact produced by an increase in the proportions of inactive molecules of the enzyme at low temperatures. This possibility would explain why the turnover seems to be lower for the old rats at lower temperatures, but it does not explain why the turnover was still lower for the old rats at higher temperatures. The found increment in the apparent energy of activation (from 10 to 20°) could be real. In this case, it is necessary to explain why the turnover for the old rats was lower than the turnover for the young rats for all the tested temperatures, whereas the energy of activation (from 20 to 52°) was unaffected by ageing. It is obvious that these possibilities do not explain the present results satisfactorily. There may be a factor that affects differentially the apparent energy of activation and the turnover of the Na^+,K^+-ATPase at a different range of temperatures.

Table 1. Apparent energies of activation of the Na^+,K^+-ATPase of basolateral plasma membranes of kidney proximal tubular cells from young (3 months) and old (24 months) rats*

Age (months)	Apparent energy of activation (K calories/mole)	
	Temperature range	
	10–20°	20–52°
3	29.82 ± 1.22	12.52 ± 0.28
24	46.10 ± 1.38	13.32 ± 0.35
Variation	+16.28 ± 1.84†	+0.80 ± 0.45‡

* The apparent energies of activation were calculated from the Arrhenius plot of the data shown in Fig. 4, by means of the following expressions: $E_a = -m \cdot 2.303 \cdot R$, where m is the slope of the Arrhenius graphic and R is the gas constant (1.987 cal·mole^{-1}·K^{-1}).

Values are expressed as mean ± S.E. of eight determinations.

† $P < 0.001$.

‡ Not significant.

Considering that (1) cholesterol has been shown to have a condensing effect on membranes, lowering their fluidity [13], and (2) it has been found that the activity of the Na^+,K^+-ATPase is inversely proportional to the cholesterol/phospholipids molar ratio of the membranes [13, 15–17], we studied the cholesterol and phospholipid contents of our membrane preparations. It was found that basolateral plasma membranes from old rat kidney cortical cells had an increased cholesterol content [from 122 ± 2 (young) to 156 ± 5 (old) nmoles/mg protein], whereas the phospholipid contents were the same [245 ± 11 (young) and 260 ± 9 (old) nmoles/mg protein]. The cholesterol content of the old rat membranes was diminished by treating them with cholesterol oxidase (from 156 ± 5 to 126 ± 3 nmoles/mg protein), without any change in the phospholipid contents. The turnover of the Na^+,K^+-ATPase was increased significantly after this treatment (for details see Ref. 18). The present results, together with the role of cholesterol in the modulation of the turnover of the Na^+,K^+-ATPase, can be interpreted as an indication that the changes produced during ageing occur in the environments of the Na^+,K^+-ATPase, i.e. as increments in condensing factors, without any change in the behaviour of the enzyme toward its ligands and the optimum pH and temperature values.

Acknowledgements—It is a pleasure to acknowledge Dr. Kent M. Chapman for his reading and criticism of this paper. Thanks are also due to Ms. Elena Suárez for her secretarial help and to Mrs. Dhuwya Otero for the drawings. The project was partially supported by CONICIT No. SI-1245.

REFERENCES

1. R. Marín, T. Proverbio and F. Proverbio, *Acta cient. Venez.* **34**, 48 (1983).
2. F. Proverbio, T. Proverbio and R. Marín, *Gerontology* **31**, 166 (1985).
3. R. E. Beauchene, D. D. Fanestil and C. H. Barrows, *J. Geront.* **20**, 306 (1965).
4. R. Marín, T. Proverbio and F. Proverbio, *Biochem. Pharmac.* **34**, 2275 (1985).
5. G. Whittembury, *J. gen. Physiol.* **48**, 699 (1965).
6. R. Marín, T. Proverbio and F. Proverbio, *Acta cient. Venez.* **34**, 322 (1983).
7. F. Proverbio and J. R. del Castillo, *Biochim. biophys. Acta* **646**, 99 (1981).
8. P. L. Jørgensen and J. C. Skou, *Biochim. biophys. Acta* **233**, 366 (1971).
9. J. S. Charnock, A. F. Almeida and R. To, *Archs. Biochem. Biophys.* **147**, 323 (1971).
10. J. S. Charnock, D. A. Cook and R. Casey, *Archs. Biochem. Biophys.* **167**, 480 (1975).
11. C. M. Grisham and R. E. Barnett, *Biochemistry* **12**, 2635 (1973).
12. K. Taniguchi and S. Iida, *Biochim. biophys. Acta* **274**, 536 (1972).
13. D. Papahadjopoulos, M. Cowden and H. K. Kimelberg, *Biochim. biophys. Acta* **330**, 3 (1973).
14. R. N. Priestland and R. Whittam, *J. Physiol. Lond.* **220**, 353 (1972).
15. W. Fiehn and D. Seiler, *Experientia* **31**, 773 (1975).
16. H. K. Kimelberg and D. Papahadjopoulos, *J. biol. Chem.* **249**, 1071 (1974).
17. D. Hegner and D. Platt, *Mech. Ageing Dev.* **4**, 191 (1975).
18. F. Proverbio, T. Proverbio and R. Marín, in *The Sodium Pump* (Eds. I. M. Glynn and J. C. Ellory). Co. Biologists, Ltd. Cambridge (1985).