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Urate and antioxidants inhibit Na^+-adenosine transport in rat renal brush border membranes

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Abstract

The effects of urate and antioxidants were evaluated on sodium-dependent [^{3}H]adenosine transport (Na^+/ADO) in rat renal brush border membrane vesicles (BBMV). Na^+/ADO was estimated for a range of adenosine concentrations of 1–10 μmol/l in BBMV preincubated with urate (0.5–50 μmol/l). Michaelis-Menten kinetics showed a significant increase in K_m values from 2.48 ± 0.49 μmol/l in control to 20.58 ± 4.56 μmol/l with 50 μmol/l urate; V_{max} (243 ± 15 pmol/mg protein $\times$ min) was not modified. Menadione (10 μmol/l) significantly increased the Na^+/ADO activity, from 17.57 ± 5.50 in the control, to 27.70 ± 7.60 pmol/mg prot. $\times$ min (a 1.60 times increase, $p < 0.05$). This stimulation was prevented when BBMV were preincubated with either 1 μmol/l α-tocopherol (trolox) or urate. Similarly conjugated dienes and malonaldehyde were stimulated in a dose-dependent fashion by menadione and the effect was inhibited with 10 μmol/l trolox. The antioxidants probucol, captopril and allopurinol inhibited in a concentration-dependent manner the Na^+/ADO (IC_{50} were 79 ± 8, 100 ± 9 and 89 ± 9 nM, respectively). This effect might be specific on K_m of the Na^+/ADO, since 1 μmol/l trolox ($IC_{50} = 1000 \pm 20$ nM), inhibited V_{max} but not K_m of the Na^+/glucose transport. Our results suggest that the Na^+/ADO in BBMV is modified by agents that affect the redox status of the membranes.

Keywords: Uric acid; Sodium-dependent adenosine transport; Antioxidants; Oxygen free species; Brush border membrane vesicles

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1. Introduction

The kidney is highly susceptible to acute hypoxia and ischemia–reperfusion injury (van Waarde et al., 1992). Under these conditions a large quantity of oxygen free radicals is produced, implicating these adducts in renal cellular insult (Baud and Ardaillou, 1986). Concomitantly, experiments in both isolated kidneys and cultured renal epithelial cells have shown a marked rise in adenosine concentrations in extracellular fluids following a brief period of hypoxia (Baud and Ardaillou, 1986; van Waarde et al., 1992; Le Hir and Castling, 1993; Baranowski and Westenfelder, 1994; Reyes et al., 1995). Due to these findings, it has been proposed that adenosine might play a role in a protective feedback mechanism to mitigate renal hypoxic damage (van Waarde et al., 1992).

A sodium-coupled adenosine transporter (Na^+/ADO) has been characterized in the luminal membrane of the proximal tubule of the rat kidney (Trimble and Coulson, 1984; Le Hir and Dubach, 1985). The net effect of this transporter is to restore the intracellular adenosine concentration in the proximal tubule epithelial cells with a concomitant fall in extracellular adenosine levels (van Waarde et al., 1992).

Uric acid has been proven to inhibit Na^+/ADO activity by approximately 40% (Trimble and Coulson, 1984).

Uric acid has traditionally been considered to be a waste product of purine metabolism (Becker, 1993). However, recent evidence suggests that it functions as an effective endogenous antioxidant (Ames et al., 1981; Becker, 1993). Urate's antioxidant activity accounts for 58% of the peroxyl radical trapping capacity of blood plasma (Uotila et al., 1992). Recently we have demonstrated that the Na^+/ADO activity is diminished in the hypothyroid rat (Martínez et al., 1997). Thyroid hormones induce superoxide radical and hydrogen peroxide production and this effect can be blocked by antioxidants (Fernández and Videla, 1993a,b). Thus, it is probable that the Na^+/ADO is regulated by the redox status of the membranes.

Our objective was to determine whether uric acid's inhibition of the Na^+/ADO in BBMV of the rat kidney was related to its antioxidant properties.

2. Materials and methods

2.1. Preparation of brush border membrane vesicles (BBMV)

The preparation of vesicles was carried out at 4°C according to the method previously described with modifications (Martínez et al., 1997). The BBMV were rehomogenized in 20 ml of intravesicular buffer: 300 mmol/l mannitol, 20 mmol/l Hepes–Tris, 50 μmol/l EHNA (erythro-9-(2-hydroxy-3-nonyl)-adenine), pH 7.4. EHNA was added to inhibit adenosine deaminase, a crucial enzyme in adenosine catabolism. The final volume of BBMV suspension was adjusted to yield a protein content of 35–50 mg/ml. The vesicles were kept in liquid nitrogen until required.

2.2. Protein determination

Protein determination was carried out according to the bicinchoninic acid method (Smith et al., 1985) using a kit assay (Pierce, USA). The standard curve was adjusted between 1 and 20 μg/ml using γ-immunoglobulin as the standard protein. Samples were 50 μl of appropriately diluted BBMV (1:100, v/v) plus 950 μl of the work solution, incubated at 60°C for 30 min. Readings were performed at 562 nm in a Shimadzu Spectrophotometer (UV-1601).

2.3. Determination of adenosine metabolism in membrane preparations

Before initiating the studies of adenosine transport, it was necessary to establish that the adenosine added during the uptake was not metabolized during incubation times.

The effectiveness of EHNA to inhibit adenosine deaminase and consequently deamination of adenosine to inosine was evaluated with adenosine deaminase concentrations ranging from 25 to 200 mU/ml at 265 nm in the absence and presence of 50 μmol/l EHNA. As shown in Fig. 1,

50 μmol/l EHNA substantially decreased the deamination of adenosine when measured under these conditions. A constant percentage of deamination is still present with this EHNA concentration (32.5 ± 10%, mean ± S.D., $n = 12$). This percentage was always subtracted from subsequent results. Thus, in all assays EHNA was added to attain a 50 μmol/l final concentration.

In order to ascertain whether labeled adenosine ([^{3}H]adenosine) was also deaminated at the same rate as non-labeled adenosine in the in vitro assay in the presence of EHNA, the deamination of [^{3}H]adenosine was estimated in brush border membrane vesicles using a microassay method for enzymes of purine metabolism (Kisaki and Sakurada, 1977).

Cellulose acetate membranes (Cellogel, Chemetron Italy) soaked in citrate buffer (0.1 M sodium citrate, pH 3.4) were placed in a LKB-Bromma (2117-Multiphor) electrophoretic chamber. Six μl of standard 5 mmol/l solutions of non-labelled adenosine, inosine, ATP, ADP and AMP were used as carriers for the radioactive solution.

Twenty μl of membrane preparations were incubated with 100 μl of the following solutions:

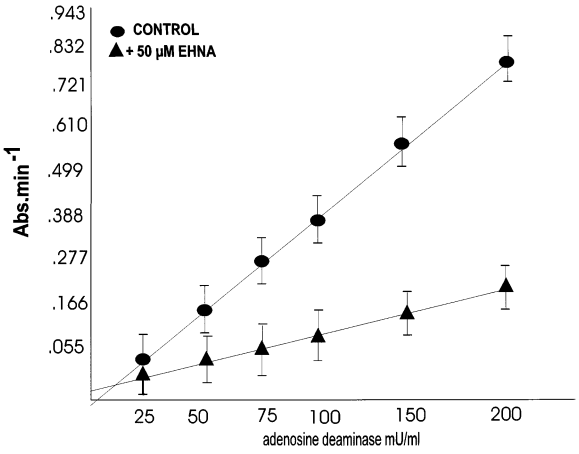

Fig. 1. Adenosine deaminase activity assay under control conditions and in the presence of 50 μmol/l EHNA. The activity of adenosine deaminase (25–250 mU/ml) was evaluated in brush border membrane vesicles (12.5 mg protein/ml) at 25°C, at a wavelength of 256 nm and the results are expressed as mean ± S.D. of absorbance per min (Abs.min^{-1}). $n = 12$ experiments from different membrane preparations.

100 mmol/l mannitol, 100 mmol/l NaCl or 100 mmol/l KCl, 20 mmol/l Hepes, 50 μmol/l EHNA, pH 7.4, and 1 μmol/l [^{3}H]adenosine, at 5, 60 and 3600 s. The reaction was stopped with 500 μl of perchloric acid and centrifuged at 59600 × g in a Beckman centrifuge (L8-50 M/E) at 4°C for 20 min. The supernatant was withdrawn and stored in liquid nitrogen until required.

The electrophoretic pattern was obtained applying 100 V for 3 h. The marker bands were identified under UV light, cut out and immersed in 5 ml of scintillation liquid (Aquasol 2, NEN-Dupont). Previously, the standard solutions had been separated independently, under identical conditions, to identify each metabolite according to its migration pattern. Uric acid was identified in a similar manner but using a different buffer (100 mmol/l Tris–HCl, pH 7.4). With this assay it is possible to measure as little as 0.4 nmol of inosine in a reaction mixture containing 10 nmol/l of adenosine. The results are expressed as a percentage of the initial label.

At 5 s, 1 μmol/l [^{3}H]adenosine recovery consisted of 85 ± 15% adenosine and 10 ± 2% inosine; at 30 s [^{3}H]adenosine recovery was 65 ± 15% adenosine and 25 ± 5% inosine; at 60 min [^{3}H]adenosine recovery was 45 ± 15% adenosine, 30 ± 15% inosine, 25 ± 5% uric acid and 5 ± 2% hypoxanthine.

2.4. Transport experiments

Prior to transport experiments, stored BBMV were thawed at 25°C and diluted with intravesicular buffer in order to have 35–60 μg of protein per sample/filter. The membranes were then resuspended by passing them, with a syringe, through a 25-gauge needle 20 times and were kept on ice until the beginning of the experiment. When the effects of uric acid and antioxidant drugs were tested, a previous 30 min incubation of BBMV with the corresponding compound was performed.

Adenosine transport was determined using the rapid filtration technique. The reaction was initiated by mixing 10 μl of BBMV with 50 μl of sodium or potassium buffer (sodium buffer: 100 mmol/l mannitol, 100 mmol/l NaCl, 20 mmol/l

Hepes–Tris, 50 μmol/l EHNA, pH 7.4; potassium buffer: 100 mmol/l mannitol, 100 mmol/l KCl, 20 mM Hepes–Tris, 50 μmol/l EHNA, pH 7.4) containing labelled adenosine (10 μCi/ml). Non-labelled adenosine was added in order to attain concentrations of 1–10 μmol/l. To accurately determine the rate of adenosine uptake, we chose 5 s because adenosine uptake remains linear up to 15 s. The equilibrium value for adenosine transport was measured at 60 min to ensure that the system was at steady state. At time intervals between 5 s and 60 min, the reaction was terminated by addition of 1 ml of ice-cold stop solution (in mmol/l: 300 mannitol, 80 Na_2SO_4, 5 Tris, 10 μmol/l dypiridamole, pH 7.4). The mixed solution was then poured immediately on to filters moistened with stop solution, and kept under suction in a Millipore device (XX2702550). The filters were then washed twice with 1 ml of ice-cold stop solution. Filtration was completed in 2–3 s. The filters were then removed and placed in vials with 5 ml scintillation fluid (Aquasol 2, NEN). The radioactivity remaining on the filters was measured in a liquid scintillation counter (1209 Wallac Rackbeta). Blank values were subtracted from the total [^{3}H]adenosine uptake, and these values were then corrected for nonspecific binding and protein content. Results are in mol/mg protein × min.

[^{3}H]Glucose transport kinetics (0.15–20 mmol/l) were measured in the presence of a 150 mmol/l NaCl outside–inside gradient in the presence and absence of 1 μmol/l of trolox. Control series for these experiments were carried out replacing Na^+ with K^+. The results are expressed as the [^{3}H]glucose uptake in the presence of Na^+ minus the [^{3}H]glucose uptake in the presence of K^+. The rest of the procedure was as explained above.

2.5. Menadione experiments

Oxidation challenges were induced with menadione treatment, which increases H_2O_2 production in both in vivo and in vitro models (Hassoun et al., 1995). BBMV were thawed at 25°C and diluted to the appropriate volume with the intravesicular buffer as indicated for transport experiments. BBMV were then incubated for 30 min with 10 μmol/l menadione. The rest of the procedure was as described for the transport experiments.

2.6. Conjugated dienes and malonaldehyde assays

After previous lipid extraction, samples were stored at −80°C and assayed for conjugated dienes according to the method reported by Lucchi et al. (1993). Lipids were suspended in a cyclohexane solution to give 0.4 mg lipid/ml solution. The lipid solution was scanned between 320 and 340 nm absorbance and a second derivative spectra was obtained in a diode-array detection spectrophotometer. Malonaldehyde was measured by the method reported by Ohkawa et al. (1979). BBMV were thawed at room temperature and mixed with sodium dodecyl sulphate, and then treated with thiobarbituric acid in acid medium and heated at 95°C for 60 min. The colored complex was extracted by butanol, and absorbance at 532 nm was determined against a standard range.

2.7. Statistical analysis

The data are presented as the mean ± standard deviation (S.D.). Experiments were repeated at least six times from different membrane preparations and each point represents assays in triplicate. Michaelis-Menten kinetics and the constants K_m and V_{max} were obtained by non-linear regression analysis using the computer program Enzfitter (Elsevier-Biosoft, UK). Results were analyzed with the SigmaStat computer program (SigmaStat 1.0, Jandel Co., 1994). The ANOVA test was used to compare differences in the effect of drugs, followed by a Dunett's test for multiple comparisons. When required, the Student's t-test was used to analyze differences between paired groups. Statistical differences with $p < 0.05$ were considered significant.

2.8. Chemicals

[^{3}H]Adenosine (60.5 Ci/mmol) was from Amersham (UK); Scintillation fluid Aquasol-2 (NEF-952, Universal LSC cocktail) was from DuPont

(USA); Pentobarbital sodium salt was from Smith Kline Beecham (Mexico); Bicinchoninic acid protein assay kit was from Pierce (USA); uric acid (2,6,8-trihydroxypurine) from Fluka Chemie AG (Switzerland); trolox (6-hydroxy-2,5,7,8-tetramethyl-croman-2-carboxylic acid) from Aldrich Chem Co. (USA). Probucol (4,4-isopropylidenedithiol-bis-(2,6-di-terbutylphenol)); captopril ([2*S*]-1-[3-mercapto-2-methylpropionyl]-L-proline)); allopurinol (4-hydroxypyrazol-3,4-pyrimidine)); menadione (vitamin K3; 2-methyl-1,4-naphthoquinone); adenosine (9-β-D-ribofuranosyl adenine) and EHNA were from Sigma (St. Louis, MO). Filters, 0.65-μM pore size (DAWP 025-00), were obtained from Millipore (USA). All other chemicals were from Merck (Germany).

3. Results

The initial rate of [^{3}H]adenosine transport (5 s) was determined at adenosine concentrations ranging from 0.5 to 10 μmol/l, whereas the effects of uric acid on adenosine transport were evaluated using uric acid concentrations ranging from 0.5 to 50 μmol/l. As shown in Fig. 2, the Na^+/ADO was inhibited by uric acid in a dose-dependent manner and completely abolished at the 10 and 50 μmol/l uric acid concentrations when tested for 10 μmol/l adenosine. The sodium-independent adenosine transport, measured in the presence of KCl buffer, was not modified by uric acid. Comparisons of different adenosine concentrations in the presence of KCl showed no statistical differences. When uric acid concentrations were increased above 50–200 μmol/l no further changes were observed in the sodium-independent adenosine transport (data not shown).

Table 1 shows the fitted Michaelis-Menten constants, K_m and V_{max}, of the Na^+/ADO in the presence of increasing concentrations of uric acid. K_m values increased significantly as uric acid concentrations increased ($p < 0.05$), whereas V_{max} did not change significantly. When fitted to Woolf–Augustinsson–Hofstee (Fig. 3) or Eadie–Scatchard plots (data not shown), the results indicated a competitive inhibition mechanism of uric acid on the Na^+/ADO. Changes in K_m values suggest an alteration in the affinity of the carrier for adenosine, without affecting the number of transporters for this nucleoside.

Since changes in affinity could be explained by modifications of the active site of the carrier or in the lipid environment of the membrane, we decided to investigate whether changes in the redox state of BBMV might be related to the activity of the transporter. Menadione, a known free radical inducer, at 10 μmol/l increased the initial rate of Na^+/ADO from 17.57 ± 5.50 to 27.70 ± 7.60 pmol/mg protein $\times$ min, a 1.6-times increase ($p < 0.05$, Fig. 4). Conjugated dienes (absorbance 320/340 nm) remained unchanged with the addition of adenosine (0.36 ± 0.38 control, vs 0.36 ± 0.020) and trolox (0.35 ± 0.023) and increased to 0.44 ± 0.44 ($p < 0.05$), 0.51 ± 0.063 ($p < 0.05$) and 0.66 ± 0.073 ($p < 0.05$) with 1, 10 and 50 μmol/l menadione, respectively (Fig. 5A). When the peroxidation product malonaldehyde was measured control values from 1.19 ± 0.095 nmol/mg protein were not modified by the addition of adenosine (1.15 ± 0.033 nmol/mg protein) or trolox (1.13 ± 0.041 nmol /protein) but increased to 1.31 ± 0.68, 1.42 ± 0.111 and 1.65 ± 0.97 nmol/mg protein ($p < 0.05$) with 1, 10 and 50 μmol/l menadione, respectively (Fig. 5B). The addition of α-tocopherol inhibited the effects of menadione in malonaldehyde production at 1 and 10 μmol/l (1.13 ± 0.026, 1.19 ± 0.016 nmol/mg protein, respectively) but not at the 50 μmol/l dose (1.45 ± 0.169, nmol/mg protein, $p < 0.05$). Conjugated dienes were inhibited in a similar manner by trolox (0.35 ± 0.023 control, vs 0.35 ± 0.018 and vs 0.39 ± 0.032 nmol/mg protein with 1 and 10 μg) but not at the highest dose (0.52 ± 0.109 nmol/mg protein, $p < 0.05$) (Fig. 5A,B). Incubation of BBMV with 1 μmol/l uric acid or α-tocopherol inhibited the Na^+/ADO ($p < 0.05$) and prevented the menadione-induced increase. This effect is specific for the K_m of the Na^+/ADO since the sodium-dependent [^{3}H]glucose transporter kinetics (0.15–20 mmol/l) were modified by 1 μmol/l trolox only on V_{max} and not on K_m (Fig. 6) ($K_m = 4.8 \pm 1.22$ in control vs 5.4 ± 2.70 mmol/l with trolox (n.s.); $V_{max} = 55 \pm 11$ in control vs 83 ± 17 nmol/mg protein $\times$ min with trolox ($p < 0.05$)).

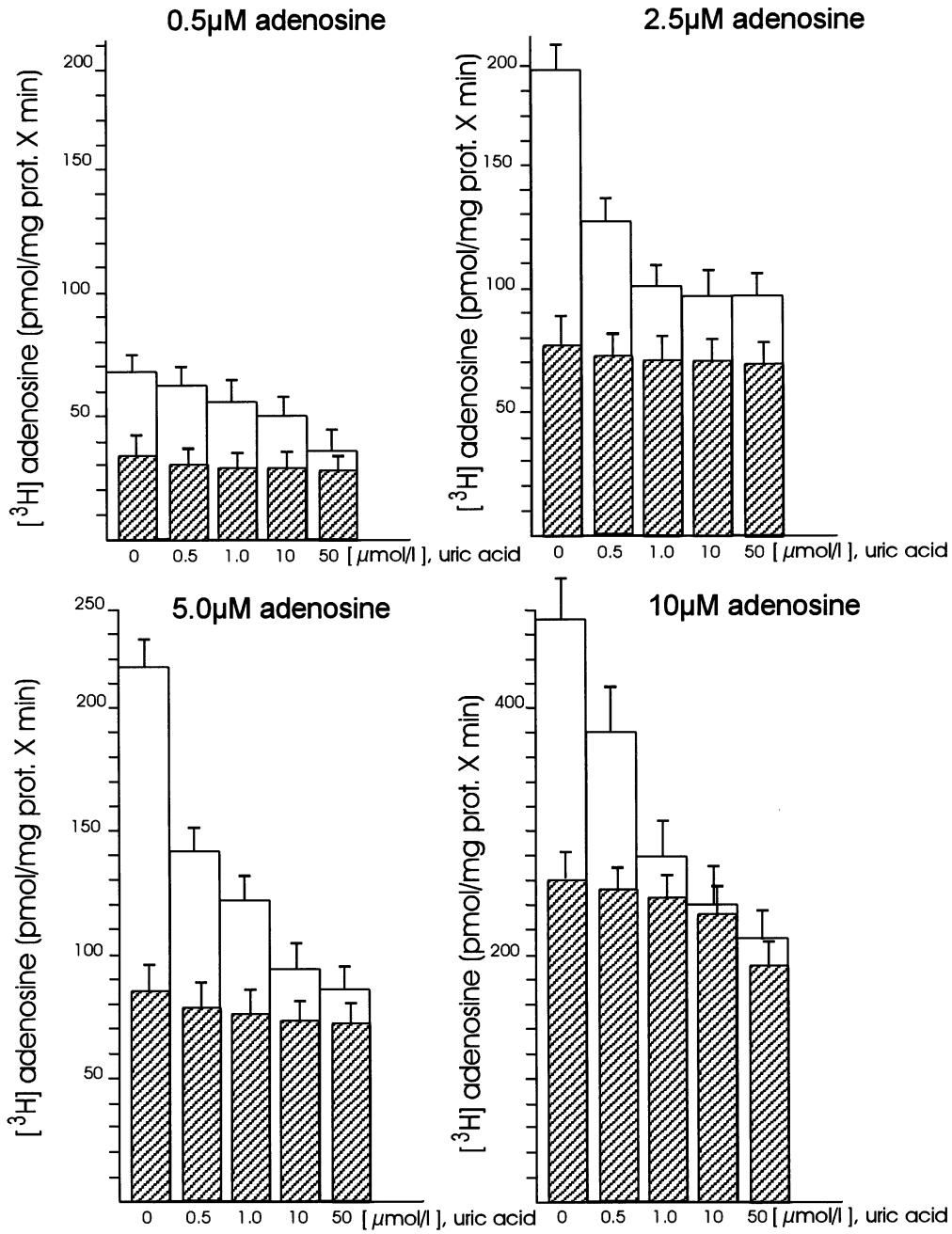

Fig. 2. Effects of uric acid (0.5 to 10 μmol/l) on Na^+/ADO in BBMV. Open bars indicate the transport activity in the presence of NaCl. Dashed bars indicate the adenosine uptake in the presence of KCl. The experiments were performed at different adenosine concentrations (0.5–10 μmol/l), and are expressed as mean $\pm$ S.D. of six experiments from different preparations.

In order to test whether the inhibitory effect of trolox was related to its free radical scavenger properties, we measured adenosine transport in the presence of the following antioxidants: allopurinol, probucol and captopril. Concentrations in the range 0.05–50 μmol/l of these drugs were

Table 1
Fitted Michaelis–Menten, K_m and V_{max}, of the Na^+/ADO in the presence of increasing concentrations of uric acid (0.02–50 μmol/l)

Uric acid (μmol/l)	K_m (μmol/l)	V_{max} (pmol/mg protein × min)
0	2.48 ± 0.49	243 ± 15
0.2	2.56 ± 0.55	254 ± 24
0.3	9.78 ± 1.80*	222 ± 17
0.5	17.59 ± 4.0*	282 ± 44
1.0	18.34 ± 5.20*	247 ± 53
10.0	23.82 ± 6.38*	292 ± 46
50.0	20.58 ± 4.56*	255 ± 43

*$p < 0.05$ vs control.

evaluated on Na^+/ADO. Fig. 7 shows the percentage inhibition by probucol (Fig. 7A), allopurinol (Fig. 7B), captopril (Fig. 7C) and trolox (Fig. 7D) on the Na^+/ADO. All four compounds inhibited adenosine transport in a concentration-dependent manner. IC_{50} values for these drugs were 79 ± 8, 89 ± 9, 100 ± 9 and 1000 ± 20 nmol/l for probucol, allopurinol, captopril and trolox, respectively.

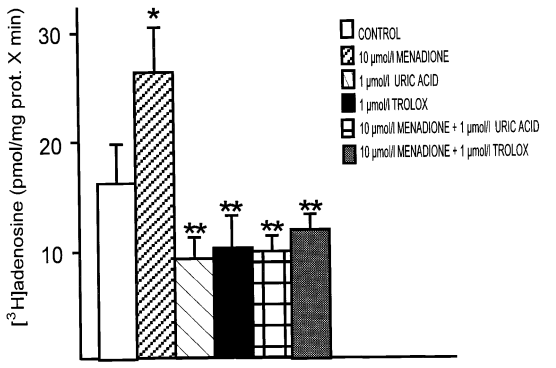

Fig. 4. Effect of menadione and the antioxidants, uric acid and trolox, on the activity of the Na^+/ADO in BBMV. The control values shown indicate the difference in the transporter activity in the presence and in the absence of KCl (as previously explained for Fig. 2). The other results were compared to the control values. The results are expressed as mean ± S.D. of six experiments from different membrane preparations. *$p < 0.05$ vs control; **$p < 0.05$ vs menadione.

4. Discussion

Our results indicate that uric acid decreased Na^+/ADO activity in BBMV from the outer renal cortex of the rat by a competitive type inhibition. This transport inhibition was probably due

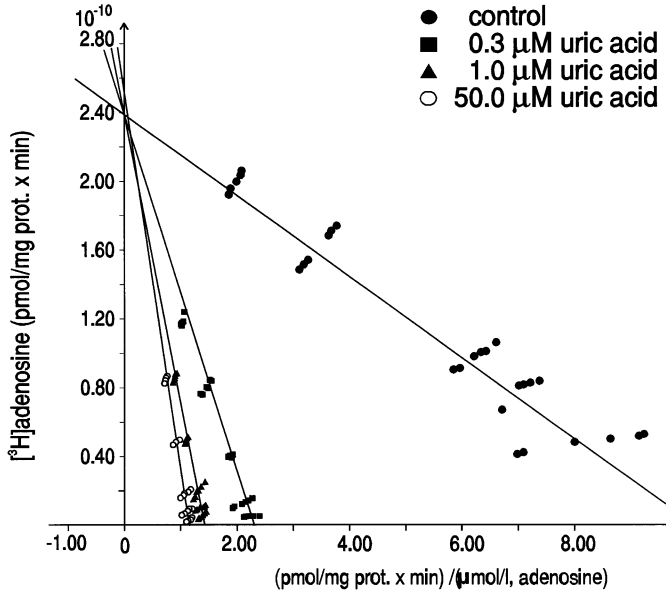

Fig. 3. Woolf–Augustinsson–Hofstee plot of the Na^+/ADO at different concentrations of uric acid (0.3–50 μmol/l). Single experiments are represented by each point in six different membrane preparations.

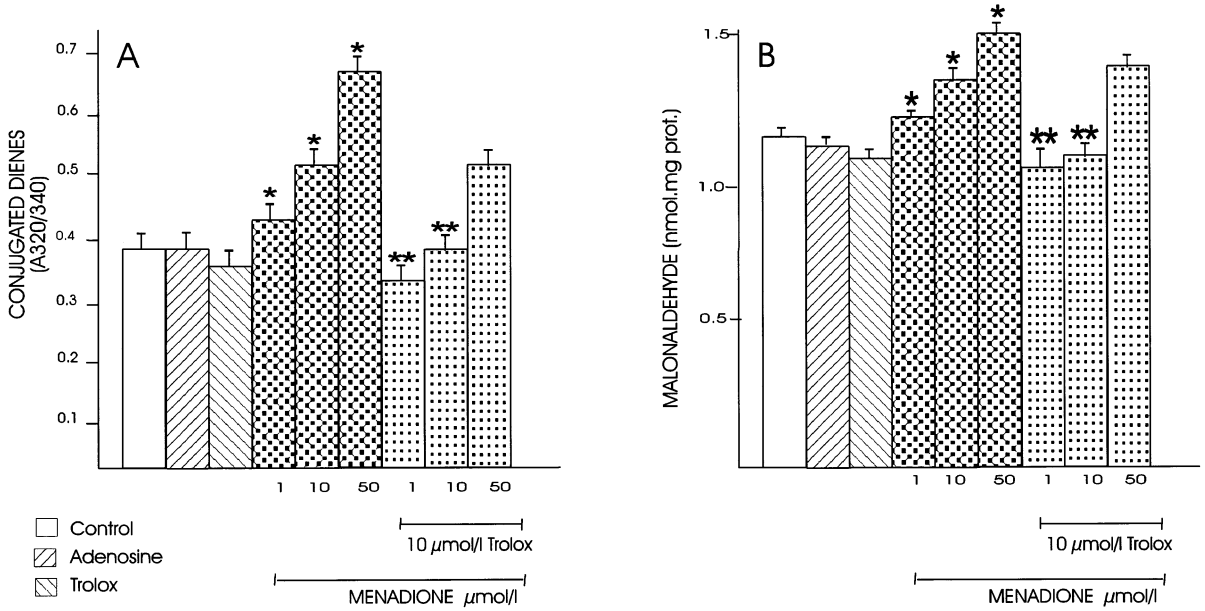

Fig. 5. Conjugated dienes (A) and malonaldehyde (B) production in response to adenosine, trolox, menadione and menadione + trolox in BBMV. $*p < 0.05$ vs control, $**p < 0.05$ vs menadione. Results are expressed as mean ± S.D. of six different membrane preparations.

to alterations in the lipid environment of the carrier caused by the free radical scavenger activity of uric acid. This mechanism is supported by our findings related to the effects of menadione, a potent free radical generator, and the antioxidant drugs tested on the transporter activity.

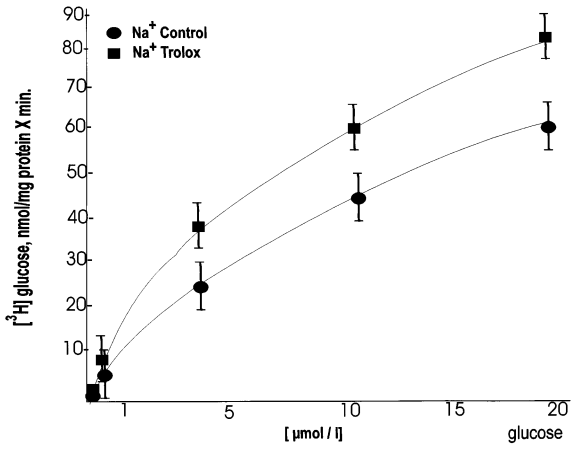

Fig. 6. Effect of α-tocopherol (trolox) on sodium-dependent glucose transport kinetics in rat BBMV. The [^{3}H]glucose uptake was measured for glucose concentrations from 0.150 to 20 mmol/l. Results are expressed as mean ± S.D. of 12 different membrane preparations.

In rat BBMV, two Na^+/ADO systems with different K_m values (2 and 43 μmol/l) have been identified (Trimble and Coulson, 1984; Franco et al., 1990). Studies in various species indicate that the low K_m system contributes to restore physiological intracellular nucleoside levels (Griffith and Jarvis, 1994). The high K_m system, on the other hand, participates in the uptake of purine analogues, such as tubercidin, N^6-methyladenosine and adenine-9-β-D-arabinofuranoside, which may explain the nephrotoxicity reported during treatment with nucleoside drugs in both humans and animals (Le Hir and Dubach, 1985). The affinity of Na^+/ADO in our model was comparable to similar preparations and it was diminished in the presence of increasing concentrations of uric acid (0.2–50 μmol/l). The sodium-independent transport was not modified by urate, even at concentrations of 50–200 μmol/l (data not shown).

A previous study (Trimble and Coulson, 1984) suggested that the affinity of the Na^+/ADO was decreased by uric acid in rat BBMV. However, this study did not distinguish whether these changes were directly on the transporter affinity

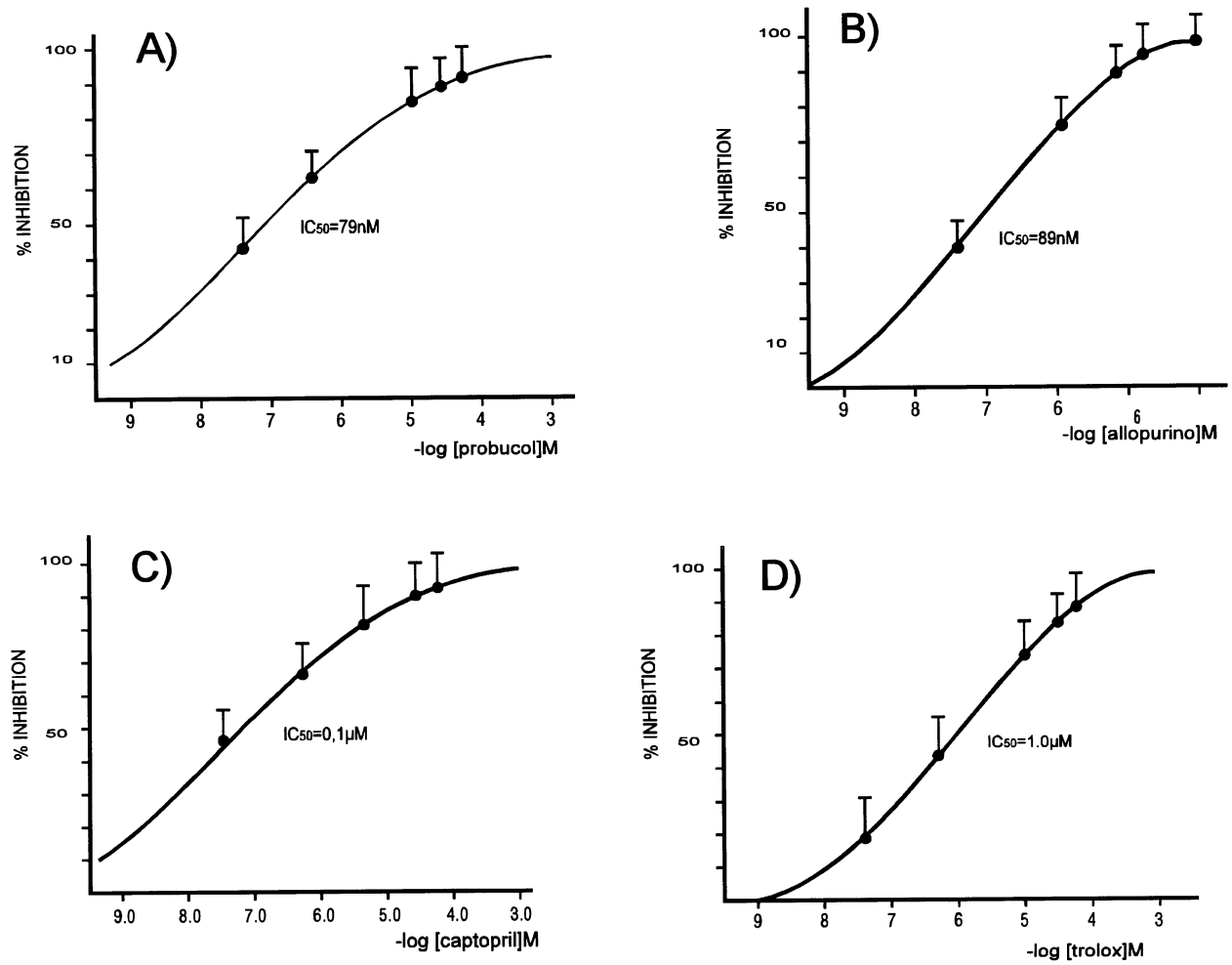

Fig. 7. Comparison of the effects of antioxidant compounds on the Na^+/ADO in BBMV. IC_{50} values for these drugs were 79 ± 3, 89 ± 6, 100 ± 4, 1000 ± 7 nM for probucol, allopurinol, captopril and trolox, respectively.

or in the membrane lipids. In some sodium-coupled carriers, alterations associated with modifications in membrane fluidity induce changes in V_{max}, as is the case of trolox on the sodium-glucose transporter in our study. Conversely, recent evidence indicates that changes in lipid environment decrease membrane fluidity and alter some carriers' affinity (K_m), without affecting the V_{max} (Imai, 1992; Courjault-Gautier et al., 1995). Such is the case of the Na^+/ADO in our work.

The kinetics of the inhibition of Na^+/ADO activity produced with uric acid clearly indicates changes in K_m values. These differences suggest an alteration in affinity of the Na^+/ADO for adenosine, without affecting the number of transporters for this nucleoside. We have recently reported a decreased Na^+/ADO activity in BBMV from the hypothyroid rat (Martínez et al., 1997). This diminished transport is related to specific changes in K_m values. Hypothyroidism alters membrane lipid content, thereby modifying membrane fluidity (Le Grimellec, 1992). Thus, we hypothesize that changes in the lipid environment due to hypothyroidism might be related to the redox status of the membrane and could induce changes in the carrier affinity.

The transport inhibition obtained with uric acid was probably due to alterations in the lipid environment of the carrier caused by the free radical scavenger activity of urate. Indeed, addition of menadione, a potent free radical generator, to the membranes increased significantly the Na^+/ADO system, and this effect was prevented by uric acid and trolox, a well-known antioxidant. This observation was further supported when we measured conjugated dienes and malonaldehyde, known products of lipid peroxidation. These products increased in a dose-dependent manner with menadione, and the effect was completely blocked by trolox, indicating that the transporter activity was directly related to inhibition of free radicals. The efficiency of menadione as a free-radical inducer has previously been evaluated in models involving transferrin and insulin receptor regulation and recycling (Malorni et al., 1993a,b).

Other antioxidants (allopurinol, probucol and captopril) also inhibited the Na^+/ADO in our study (Fig. 3), supporting the notion that lipid peroxidation affects transporter activity. The inhibition of lipid peroxidation and peroxidative index produced by these drugs is not doubtful (Fukuzawa et al., 1993; Kuzuya and Kuzuya, 1993; Misk et al., 1993; van Acker et al., 1993; Mira et al., 1994; Reguli and Misik, 1994; Jay et al., 1995; Mabile, 1995).

It is probable that probucol and trolox (a hydrosoluble α-tocopherol) play significant roles in the inhibition of lipoperoxidation and consequently decrease the Na^+/ADO activity. Probucol is a highly lipophilic compound that gets actively incorporated into the cellular membranes of myocytes, erythrocytes and endothelial cells and prevents their lipoperoxidation (Eisenberg, 1993; Kuzuya and Kuzuya, 1993). Similarly, tocopherols are retained in lipid bilayers, with the OH group of the aromatic ring oriented towards the surface (Mabile, 1995). This phenolic hydroxyl group traps peroxyl radicals as they emerge from the membrane core (Fukuzawa et al., 1993; van Acker et al., 1993; Mabile, 1995).

Captopril, an angiotensin-converting enzyme (ACE) inhibitor, is also a potent free radical scavenger. This action requires the presence of thiol groups (Misk et al., 1993; Mira et al., 1994) and mimics superoxide dismutase activity in the presence of copper ions and transition metals (Reguli and Misik, 1994; Jay et al., 1995).

Allopurinol is a substrate and potent inhibitor of the enzyme xanthine:NAD^+ oxidoreductase (XDO) (Komoriya, 1993). This is the final enzyme in the catabolic pathway for purines and catalyzes the production of uric acid from hypoxanthine and xanthine. Superoxide radicals are formed by the single-electron reduction of oxygen by xanthine oxidase during hypoxia and ischemia–reperfusion injury (Komoriya, 1993). Allopurinol inhibits this mechanism (Day et al., 1994). Isolated nephron segments and BBMV exhibit XDO activity (Ha and Endou, 1992). Increased enzyme activity may result in a high rate of superoxide radical production and consequently in lipid peroxidation (Hise et al., 1984).

To evaluate the specificity of antioxidants on the Na^+/ADO, we also studied the effect of trolox on the sodium-dependent glucose transport, a characteristic carrier of the proximal tubule. This transporter is highly sensitive to specific hormone regulation and also to inhibition by specific and non-specific compounds, and thus is frequently used as an indicator of BBMV functionality and for testing the selectivity of drug effects (Leiser and Molitoris, 1993). Indeed, the sodium-dependent glucose transport activity was modified by trolox, specifically affecting the V_{max}, with no effect on K_m.

In conclusion, we demonstrated that uric acid inhibited Na^+/ADO in BBMV of the outer renal cortex of the rat by its free radical scavenger activity. The effects of menadione, a potent free radical generator, were diminished by both uric acid and trolox. The use of known antioxidants (probucol, captopril and allopurinol) and the evaluation of lipid peroxidation confirms our findings.

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