ELSEVIER

Biochimica et Biophysica Acta 1191 (1994) 219–224

BBA Biochimica et Biophysica Acta

Developmental changes of renal brushborder membrane ionic permeability

Marvin S. Medow [a,*], Michael S. Lipkowitz [b]

[a] Department of Pediatrics, Division of Pediatric Gastroenterology and Nutrition, New York Medical College, Valhalla, New York, NY 10595, USA, [b] Department of Medicine, Division of Nephrology, Mount Sinai School of Medicine, New York, NY, USA

(Received 2 December 1993)

Abstract

Renal brush-border membrane vesicles (BBMV) show an age-dependent increase in height of the Na^+-gradient driven overshoot for glucose and proline uptake. Conversely, early uptake of $^{22}Na^+$ is more rapid in BBMV from kidney of 7-day-old vs. adult rats. To understand the mechanisms responsible for these observations, ionic permeability characteristics of BBMV from different aged animals were determined using an electrical potential sensitive fluorescent dye, diS-C_3(5). Absolute and relative ionic permeabilities were determined after a 3-h incubation in 100 mM KCl. Intravesicular K^+ ($[K^+]_{in}$), a measure of absolute K^+ permeability, was calculated from the extravesicular K^+ at which valinomycin produced no potential difference (PD). $[K^+]_{in}$ was significantly lower in vesicles from 7-day, compared to adult ($P < 0.01$). While Cl^- permeability, relative to that of K^+ (P_{Cl^-}/P_{K^+}) was similar, P_{Na^+}/P_{K^+} decreased significantly with age ($P < 0.05$, 7 day vs. adult). In the presence of an inwardly directed NaCl gradient, the lower P_{Na^+} relative to P_{Cl^-} of the adult vesicles would result in a less positive intravesicular charge, which would therefore augment Na^+-solute co-transport. Fluorescence polarization studies also show that lipids from BBM vesicles of 7-day-old rats are more fluid than those from adult. These differences are likely due to developmental lipid compositional changes, which influence membrane transport and permeability characteristics. These findings would explain, in part, the age-dependent alterations of renal BBMV solute transport.

Key words: Renal; Brush-border membrane vesicle; Ionic permeability; Fluorescence polarization; Membrane fluidity

1. Introduction

Maturation of the mammalian kidney is accompanied by many changes, including increases in renal mass, glomerular filtration and development of renal enzyme systems [1]. Postnatal development of the nephron reduces the urinary excretion of amino acids and glucose in the rat [2], dog [3], and man [4], resulting in resolution of the so-called neonatal aminoaciduria and glycosuria. Previous studies of the ontogeny of renal transport have relied upon the use of renal cortical slices [5,6] and isolated renal tubule fragments obtained from different aged animals [7,8]. These showed that older animals achieved higher intracellular sugar and amino acid concentrations when compared to young. More recent studies, using renal brush-border membrane vesicles prepared from different aged animals, show an age-dependent increase in Na^+-dependent uptake of sugars and amino acids, resulting in an increased height of the Na^+-solute overshoot [1,9].

One factor affecting the height of the Na^+-solute overshoot is membrane potential; in brush-border membrane vesicles (BBMV) the relative permeabilities of anions and cations determine the magnitude of this potential difference (PD). Thus, alterations in relative permeabilities would modify Na^+-solute cotransport. In a previous study, examination of Na^+ transport suggested that ionic permeability changes as animals mature since $^{22}Na^+$ entry was more rapid in membrane vesicles prepared from younger animals [9]. We have also previously shown that the ionic permeability

* Corresponding author. Fax: +1 914 9934351.

SSDI 0005-2736(93)E0394-P

of Na^+, relative to that of Cl^-, is significantly lower in brush-border membranes from adult compared to 7-day-old animals [10]. A more thorough description of renal brush-border ionic permeability characteristics, comparing 7-day-old to adult animals, forms the basis for this report.

2. Materials and methods

Isolation and preparation of membrane vesicles

Gravid female Sprague-Dawley rats were obtained from Charles River (Kingston, NY) at 14 days of gestation and fed ad libitum on Purina rat chow until parturition. Pups were killed by decapitation at 7 days of age and the kidneys surgically removed and placed in ice-cold buffered saline. Male Sprague-Dawley rats > 90-days-old and designated adult (weighing 150–200 g) were obtained from the same source and used for preparation of adult brush-border membrane vesicles (BBMV). Renal BBMV from 7-day-old and adult rats were prepared utilizing a modified method of Pockrandt-Hemstedt, as previously described [11]. Renal cortical slices from 7-day-old rat kidneys were cut with a scalpel and from adult with a Stadie-Riggs microtome, the tissue homogenized in 250 mM sucrose, 12.5 mM triethanolamine, adjusted to pH 7.4 with HCl (ST buffer), and then subjected to differential centrifugation. The final pellet was resuspended in 300 mM mannitol, 10 mM Tris-Hepes, adjusted to pH 7.4 by addition of Tris (MTH buffer) to a protein concentration of ~ 5–10 mg/ml. Protein was determined by the method of Lowry et al. [12].

The purity of the final brush-border preparations was assessed by determining enrichment, relative to the starting homogenate, of alkaline phosphatase (EC 3.1.3.2) and Na^+/K^+-stimulated ATPase (EC 3.6.1.3) as previously described [9,11]. Enrichment (ratio of brush-border to homogenate enzyme activity) of alkaline phosphatase ranged from 6 to 9-fold, while Na^+/K^+-ATPase enrichment was less than 1, and there were no differences in enrichment for each enzyme comparing both age groups. Equilibrium uptake of radioactively labeled proline in vesicles from these two age groups was the same, indicating that both vesicle populations had a similar intravesicular volume [9].

Loading of vesicles with KCl

A concentrate of KCl (1100 mM), estimated to yield a final KCl concentration of 100 mM, was added to aliquots of BBMV prepared as described above. Vesicles were preincubated (loaded) in this medium for 3 h at room temperature (22°C) and then stored on ice. In each experiment, control (unloaded) vesicles, not exposed to KCl, were otherwise treated identically to loaded vesicles.

Fluorescence measurements

The positively charged fluorescent dye 3,3′-dipropylthiadicarbocyanine iodide (diS-C_3(5), Molecular Probes, Junction City, OR), a membrane potential (PD)-sensitive probe, was used to determine intravesicular electrolyte concentrations and relative ionic permeabilities as previously described [13,14]. Fluorescence was measured in a Perkin-Elmer spectrofluorophotometer (model 650–10 S) using excitation and emission wavelengths of 622 and 665 nm, respectively.

Aliquots of vesicles (100 μg of protein) were diluted into 1 ml of media containing 3 μM diS-C_3(5), 0.5–100 mM K^+, 99.5–0 mM choline, 100 mM Cl^-, and 300 mM MTH. Constant ionic strength and osmolarity were maintained to avoid artifacts in fluorescence due to changes in ionic strength, and intravesicular volume [15]. Initial fluorescence was recorded, after which the potassium ionophore valinomycin (final concentration 3 μM) was added and the resulting change in fluorescence recorded. The magnitude of any nonspecific valinomycin-induced change in fluorescence was determined by addition of valinomycin to media containing unloaded vesicles (100 μg of protein) in 100 mM choline Cl, 100 mM MTH, and 3 μM diS-C_3(5). All data were corrected for this nonspecific fluorescent effect.

The intravesicular KCl concentration was determined using the 'null point' method [13,14]. When intravesicular and extravesicular potassium are in electrochemical equilibrium, valinomycin will induce no change in PD or fluorescence. The null point was determined by calculating the $[K^+]_{out}$ at the intersections of the regression lines for initial fluorescence (fitted to the Goldman-Hodgkin-Katz constant field equation) and fluorescence post-valinomycin (fitted to the Nernst equation). After $[K^+]_{in}$ was determined, fluorescence was converted to PD in millivolts. Using PD, the ionic permeabilities of Cl^- and choline relative to K^+ were determined from the coefficient of the regression line for initial fluorescence as previously reported [13,14].

The ionic permeabilities of sodium, lithium, and gluconate relative to that of potassium were determined from the membrane potential generated by diluting KCl-loaded vesicles into media containing 3 μM diS-C_3(5), 100 mM MTH, and either 100 mM NaCl, LiCl, or K^+ gluconate, respectively. After converting the initial fluorescence to PD, P_{Na^+}/P_{K^+}, P_{Li^+}/P_{K^+} and $P_{gluconate}/P_{K^+}$ were calculated from the constant field equation by substituting the concentration of Na^+ or Li^+ for K^+, and gluconate for Cl^- in the constant field equation.

Liposome preparation

Membrane lipids were extracted from 7-day-old and adult brush-border membrane vesicles according to the method of Folch, as previously described [16]. Lipo-

somes were prepared from extracted N_2-dried membrane lipids, after suspension of lipids in phosphate-buffered saline containing the lipid-soluble probe, 1,6-diphenyl-1,3,5-hexatriene (DPH). Lipid suspensions were then sonicated under N_2 for 10 min at 4°C, and the resultant liposome dispersion was centrifuged at $10\,000 \times g$ for 10 min. The supernatant was then used for fluorescence polarization studies.

Fluorescence polarization studies

Membrane lipid fluidity was calculated following fluorescence polarization measurements using a Shimadzu RF-540 spectrofluorophotometer fitted with a thermoregulated sample chamber (Perkin-Elmer, Norwalk, CT) and automatic polarizers (C.N. Wood, Newtown, PA), as previously described [11]. Steady-state fluorescence anisotropy r (reciprocal of fluidity) were determined using the equation:

$$r = (I_{\parallel} - I_{\perp})/(I_{\parallel} + 2I_{\perp})$$

where $I_{\parallel}$ and $I_{\perp}$ equal fluorescence intensities parallel and perpendicular, respectively, to the excitation plane. Scattered light plus ambient medium fluorescence contributed $< 5\%$ to the total fluorescence intensity throughout the temperature range utilized in all studies. Constancy of fluorescence probe lifetimes was assessed from measurements of total fluorescence intensity F, where $F = I_{\parallel} + 2I_{\perp}$ [17]. This value did not vary with temperature, or among membrane/liposome preparations (comparisons by analysis of variance).

Statistical analysis

All experiments were performed on at least three membrane vesicle preparations prepared on separate occasions. In each experiment, studies were performed at least in triplicate, and the mean of these multiple determinations served as one value for the calculation of the mean of all experiments. All data are expressed as the mean ± S.E., and were evaluated using either paired or unpaired analysis. Statistical significance was determined with Student's t-test.

3. Results

Fig. 1 depicts typical experiments in which $[K^+]_{in}$ was determined in KCl loaded brush-border membrane vesicles from 7-day-old and adult rats. When vesicles from either age group were diluted into media identical to that in which they had been preincubated (100 mM KCl, 100 mM MTH; $\log[K^+]_{out} = 2$), the addition of valinomycin resulted in the generation of a significant inside-positive membrane potential. This phenomenon was observed in all seven 7-day-old and adult vesicle preparations examined. This indicates that an inwardly-directed $[K^+]$ gradient was maintained across

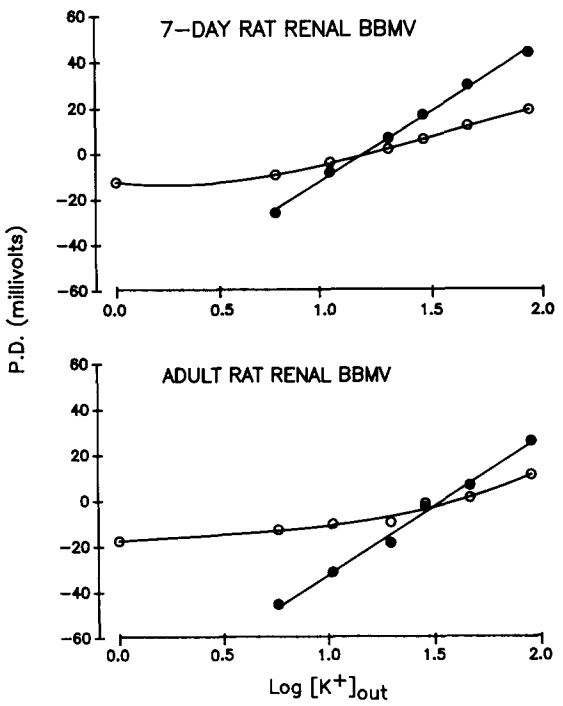

Fig. 1. Determination of transvesicular membrane potential (PD) across renal brush-border membrane vesicles from 7-day-old (upper panel) and adult rats (lower panel). (○), PD in the absence of valinomycin, (●), PD in the presence of valinomycin. Intersection of the two regression lines (null point) occurs when intravesicular and extravesicular $[K^+]$ are equal and PD is zero.

both 7-day-old and adult membrane vesicles. Fig. 2 shows that the $[K^+]_{in}$ achieved was significantly less than 100 mM in brush-border membrane vesicles from both adult and 7-day-old rats. In addition, in each experiment, $[K^+]_{in}$ was significantly lower in 7-day-old compared to adult membrane vesicles, (19.7 ± 4.3 vs. 39.9 ± 1.0, $P < 0.01$).

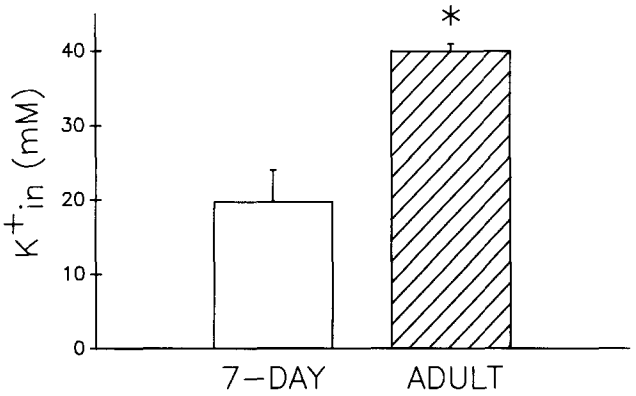

Fig. 2. Intravesicular K^+ concentration ($[K^+]_{in}$) in renal brush-border membrane vesicles from 7-day-old (open bar) and adult (hatched bar) rats. $[K^+]_{in}$ was determined using the 'null point' method, as described in Materials and methods. * $[K^+]_{in}$ was significantly lower in 7-day-old vs. adult rat renal membrane vesicles, $P < 0.01$, $n = 4$.

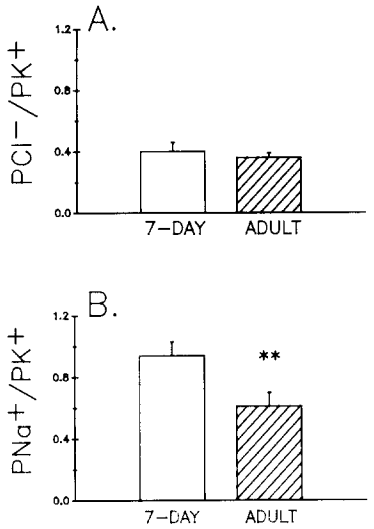

Fig. 3. Calculated permeability of chloride relative to potassium (P_{Cl^-}/P_{K^+}, panel A), and permeability of sodium relative to potassium (P_{Na^+}/P_{K^+}, panel B), in renal brush-border membrane vesicles from 7-day-old (open bar) and adult (hatched bar) rats. ** P_{Na^+}/P_{K^+} was significantly lower in adult vs. 7-day-old rat renal brush-border membrane vesicles, $P < 0.05$, $n = 4$.

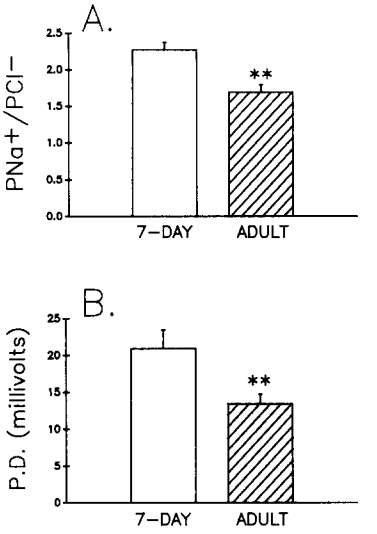

Fig. 4. Calculated permeability of sodium relative to chloride (P_{Na^+}/P_{Cl^-}, panel A), and the transvesicular potential difference (PD, panel B), in the presence of an inwardly directed 100 mM NaCl gradient, across renal brush-border membrane vesicles from 7-day-old (open bar) and adult rats (hatched bar). ** P_{Na^+}/P_{Cl^-}, and PD were each significantly lower in adult vs. 7-day-old rat renal brush-border membrane vesicles, $P < 0.01$, $n = 4$.

These findings indicate, as in previous studies of adult rats, that KCl gradients are maintained across BBMV despite prolonged incubation in 100 mM KCl. Furthermore, the KCl permeability of membrane vesicles from 7-day-old rats is significantly less than that of adult. Examination of Fig. 1 also shows a significant inside positive PD in vesicles diluted into 100 mM KCl. This implies that in the presence of an inwardly directed KCl gradient, there is a greater restriction to the transmembrane movement of chloride than potassium in these vesicles.

The calculated permeability of chloride relative to potassium (P_{Cl^-}/P_{K^+}), shown in Fig. 3A, confirms this supposition. There was however no difference between the P_{Cl^-}/P_{K^+} of vesicles from 7-day-old and adult rats. The increased intravesicular concentration of potassium in the adult suggests increased vesicular KCl permeability, relative to 7-day-old vesicles. The finding of no difference between the P_{Cl^-}/P_{K^+} of vesicles from 7-day-old and adult rats therefore suggests that the difference of $[K^+]_{in}$ between 7-day-olds and adults represents an age-dependent change in membrane permeability to both potassium and chloride. As shown in Fig. 3B, the permeability of sodium relative to potassium (P_{Na^+}/P_{K^+}), was significantly greater in 7-day-old compared to adult membranes (0.94 ± 0.09 vs. 0.61 ± 0.09, $P < 0.05$).

Since there was no difference in P_{Cl^-}/P_{K^+}, the P_{Na^+}/P_{Cl^-}, shown in Fig. 4A, was also significantly higher in vesicles prepared from younger animals. As a result of this greater permeability, the calculated PD across vesicles from 7-day-old rats was significantly greater than that across vesicles from adult rats in the presence of an inwardly directed 100 mM NaCl gradient (Fig. 4B). These differences are specific for Na^+, as the permeabilities of lithium and gluconate were the same for 7-day-old and adult BBMV (data not shown).

Previous studies have shown an ontogenic decrease in membrane fluidity of rat renal brush-border membranes [11]. Fluidity is a relative term which describes the hinderance to motion of a lipid soluble probe within the membrane bilayer; and it is influenced by membrane proteins and lipids. To distinguish between

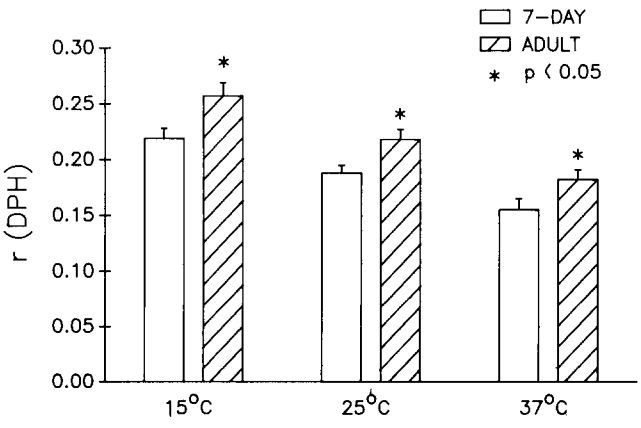

Fig. 5. Fluorescence anisotropy, r(DPH) (reciprocal of fluidity), of liposomes prepared from extracted renal brush-border membrane lipids from 7-day-old (open bar) and adult rats (hatched bar). * Lipid fluidity in liposomes from 7-day-old rats was significantly greater ($P < 0.05$, $n = 4$) compared to adults at the three temperatures shown, and for all values measured from 40 to 5°C.

protein vs. lipid effects, liposomes were prepared from lipids which were extracted from renal brush-border membrane vesicles from different aged rats, and fluorescence polarization studies performed.

Fig. 5 shows the results of these studies comparing a measure of membrane fluidity, r, on the Y-axis, at 3 different temperatures, in liposomes from 7-day and adult. Higher values of r indicate lower membrane fluidity. At these three temperatures, and all others measured between 5 and 40°C, liposomes from 7-day-old rats, were more fluid than those from adults. This suggests that differences in lipid composition of the various age groups are responsible in part, for the fluidity changes and transport maturation previously described in intact membrane vesicles.

4. Discussion

The finding (Fig. 1) that membrane vesicles from both 7-day-old and adult rats exhibited an initial fluorescence higher than that recorded at the null point, and correlation of fluorescence and PD, suggest that an inside-positive potential is present across the vesicles in the absence of valinomycin. Since the only electrolytes present in the incubation media were K^+ and Cl^-, this positive PD must be due to a potassium diffusion potential.

A comparison of intravesicular potassium concentrations in different membrane populations revealed that $[K^+]_{in}$ determined for adults was the same whether renal brush-border membrane vesicles were prepared using differential centrifugation or free-flow electrophoresis [13]. The $[K^+]_{in}$ value of 19.7 mM for vesicles from 7-day-old rats is the same as we have reported for the basolateral membranes from adult rats [14]. This suggests a relationship between membrane physical state and ionic permeability; fluorescence polarization studies have shown that brush-border membranes from 7-day-old rats and basolateral membranes from adult rats are more fluid than brush-border membranes from adult rats [11,18].

Brush-border and basolateral membranes of the proximal tubule cell are populated by a diverse array of transport proteins. It is therefore difficult to generalize about the effects of changes in membrane physical properties on transport across these membranes. However, recent data indicate that each membrane contains both K^+ and Cl^- channels [19,20] whose function and/or insertion into the cell membrane may be limited by increased fluidity. This mechanism may be responsible, at least in part for the decreased KCl permeability of 7-day-old brush-border and adult basolateral membranes, relative to the permeability of the adult brush-border membranes, which are less fluid.

Previous studies [9] have shown that the uptake of proline by renal brush-border membrane vesicles increases with age, as measured by the height of Na^+-solute overshoot. These differences have already been shown to be due in part to an increased vesicular uptake of isotopic Na^+ in 7-day-old rats, presumably via a diffusional pathway. This increased Na^+ permeability has been demonstrated fluorometrically in the present study, as has the finding that vesicles from 7-day-old rats have a significantly greater intravesicular positivity, compared to adults. Since Na^+-proline cotransport is electrogenic, resulting in the movement of a positive charge into the vesicles, the greater positive P.D. measured in the 7-day-old vesicles would restrict Na^+-proline cotransport, and may explain the age-dependent increases in vesicular proline transport.

The demonstration of membrane fluidity changes with age, both in native membranes and membrane lipids may in part explain the maturational differences in solute and ion transport, as well as enzymatic activities. These fluidity and functional alterations are due to ontogenic changes previously described in membrane lipid composition [11,21–23]. These include a maturational reduction of total membrane phospholipids, a decrease in the ratio of saturated to unsaturated fatty acids, and an increase of the membrane cholesterol to phospholipid ratio.

In summary, using fluorometric techniques, we have demonstrated that there is an increased permeability of Na^+ relative to Cl^- in brush-border membrane vesicles prepared from 7-day-old rats, compared to adults. This increased relative permeability results in an increased inside positive membrane potential in the 7-day-old compared to adult renal BBM vesicles. The greater inside positive potential would secondarily inhibit electropositive Na^+-solute cotransport processes. These differences are associated with, and perhaps in part caused by the membrane compositional changes which accompany development, and result in the demonstrated fluidity changes, permeability differences, and altered vesicle solute transport characteristics.

5. Acknowledgements

This work was supported by Grants from the American Heart Association, New York State Affiliate (M.S.M.) No. 91-020G, and National Institutes of Health (M.S.M.), No. HL 35739, (M.S.L.), No. DK 01856.

6. References

[1] Beck, J.C., Lipkowitz, M.S. and Abramson, R.G. (1988) J. Clin. Invest. 82, 163–167.

[2] Baerlocker, K.E., Scriver, C.R and Mohyddin, F. (1970) Proc. Natl. Acad. Sci. USA 65, 1009–1016.
[3] Blazer-Yost, B. and Jezyk, P. (1979) Am. J. Vet. Res. 40, 832–838.
[4] Brodehl, J. (1976) in Amino Acid Transport and Uric Acid Transport (Sibernagl, S., Lang, F. and Greger, R., eds.), George Thieme, Stuttgart, pp. 128–136.
[5] Rosenberg, L.E. and Segal, S. (1964) Biochem. J. 92, 345–352.
[6] Segal, S., Rea, C. and Smith, I. (1971) Proc. Natl. Acad. Sci. USA 68, 372–376.
[7] Hwang, S.M., Serabian, M.A., Roth, K.S. and Segal, S. (1983) Pediatr. Res. 17, 42–46.
[8] Medow, M.S., Foreman, J.W., Bovee, K.C. and Segal, S. (1982) Biochim. Biophys. Acta 693, 85–92.
[9] Medow, M.S., Roth, K.S., Goldmann, D.R., Ginkinger, K.S. Hsu, B.Y. and Segal, S. (1986) Proc. Natl. Acad. Sci. USA 83, 7561–7564, 1986.
[10] Medow, M.S. and Lipkowitz, M.S. (1989) Proc. of the 4th International Workshop on Developmental Renal Physiology (Abstract).
[11] Medow, M.S. and Segal, S. (1987) Biochim. Biophys. Res. Commun. 142, 849–856.
[12] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275.
[13] Lipkowitz, M.S. and Abramson, R.G. (1987) Am. J. Physiol. 252, F700–F711.
[14] Lipkowitz, M.S. and Abramson, R.G. (1989) Am. J. Physiol. 256, F18–F28.
[15] Wright, S.H., Krasne, S., Kippen, I. and Wright, E.M. (1981) Biochim. Biophys. Acta 640, 767–778.
[16] Schwarz, S.M., Lambert, A.S. and Medow, M.S. (1992) Biochim. Biophys. Acta 1107, 70–76.
[17] Gerritsen, M.E., Schwarz, S.M. and Medow, M.S. (1991) Biochim. Biophys. Acta 1065, 63–68.
[18] Molitoris, B. and Hoilen, C. (1987) J. Membr. Biol. 99, 165–172.
[19] Palmer, L.G. (1992) Handbook of Physiology: Renal Physiology (Windhager, E.E., ed.), Oxford University Press, New York, pp. 715–738.
[20] Wang, T.A., Segal, G., Giebisch, G. and Aronson, P.S. (1993) J. Am. Soc. Nephrol. 4, 883A.
[21] Chesney, R.W., Gusowski, N., Zelikovic, I. and Padilla, M. (1986) Pediatr. Res. 20, 890–894.
[22] Hise, M.K. and Weinman, E.J. (1986) Pflugers Arch. 406, 234–236.
[23] Levi, M., Jameson, D.M. and Wieb Van Der Meer, B. (1989) Am. J. Physiol. 256, F85–F94.